REPORT OF THE
RETINAL AND CHOROIDAL DISEASES PANEL

Volume Two/Part One

VISION research

A NATIONAL PLAN

1983-1987

vol 2
part 1

U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
Public Health Service
National Institutes of Health


M.C. MIGEL MEMORIAL LIBRARY
American Foundation for the Blind
15 West 16th Street, New York, New York
10011

REPORT OF THE
RETINAL AND CHOROIDAL DISEASES PANEL Volume Two/Part One

VISION research

A NATIONAL PLAN 1983-1987

U.S. DEPARTMENT OF HEALTH
AND HUMAN SERVICES
Public Health Service
National Institutes of Health

NIH Publication No. 83-2471

HV2332
V825
1983
Vol. 2
part 1

PREFACE

THIS IS THE Report of the Retinal and Choroidal Diseases Panel, which is Part One of *Volume Two, Reports of the Program Panels* of the multivolume report of the National Advisory Eye Council entitled, *Vision Research—A National Plan: 1983–1987.*

The complete National Plan presents a comprehensive and detailed assessment of the current NEI program as well as specific recommendations for program development over the next five years. These include program priorities and projections of resource requirements for each major area of vision research that the NEI supports. Readers desiring additional information should consult the following volumes:

Executive Summary (Overview of the entire Plan).

Volume One—The 1983 Report of the National Advisory Eye Council (Background, Summary Panel Reports and Resource Requirements, Implementation Strategy, Cross-Cutting Research Areas and Issues, Planning Participants, Planning Strategy and Process).

Volume Two—Reports of the Program Panels

Part One—*Report of the Retinal and Choroidal Diseases Panel*

Part Two—*Report of the Corneal Diseases Panel*

Part Three—*Report of the Cataract Panel*

Part Four—*Report of the Glaucoma Panel*

Part Five—*Report of the Strabismus, Amblyopia, and Visual Processing Panel*

Part Six—*Report of the Panel on Visual Impairment and Its Rehabilitation.*

Volume Three—Support for Vision Research (Data on vision research projects supported by the NEI in FY 1981 and by other government and private organizations in 1980).

Digitized by the Internet Archive
in 2011 with funding from
Lyrasis Members and Sloan Foundation

http://www.archive.org/details/visionresearchna21nati

CONTENTS

PANEL MEMBERS & CONSULTANTS

Co-Chairmen

HARRIS RIPPS, Ph.D.
Professor of Ophthalmology, Physiology, and Biophysics
New York University Medical Center
New York, New York

WILLIAM TASMAN, M.D.
Co-Director Retina Service
Wills Eye Hospital and
Professor of Ophthalmology
Jefferson Medical College
Thomas Jefferson University
Philadelphia, Pennsylvania

Members

DANIEL M. ALBERT, M.D.
Director, Eye Pathology Laboratory
Massachusetts Eye and Ear Infirmary and Professor of Ophthalmology
Harvard Medical School
Boston, Massachusetts

ELIOT L. BERSON, M.D.
William F. Chatlos Professor of Ophthalmology
Harvard Medical School and
Director, Berman-Gund Laboratory for the Study of Retinal Degenerations
Harvard Medical School
Massachusetts Eye and Ear Infirmary
Boston, Massachusetts

DEAN BOK, Ph.D.
Professor of Anatomy
Jules Stein Eye Institute
School of Medicine
University of California
Los Angeles, California

RICHARD A. CONE, Ph.D.
Professor of Biophysics
Thomas C. Jenkins Department of Biophysics
Johns Hopkins University
Baltimore, Maryland

JOHN E. DOWLING, Ph.D.
Professor of Biology
Faculty of Arts and Sciences
Biological Laboratories
Harvard University
Boston, Massachusetts

JAY M. ENOCH, Ph.D., O.D.
Professor and Dean
School of Optometry
University of California
Berkeley, California

ROBERT N. FRANK, M.D.
Professor of Ophthalmology
Kresge Eye Institute
School of Medicine
Wayne State University
Detroit, Michigan

H. MACKENZIE FREEMAN, M.D.
Vice President and Clinical Scientist
Eye Research Institute of Retina Foundation
Boston, Massachusetts

G. RICHARD O'CONNOR, M.D.
Director, Francis I. Proctor Foundation for Research in Ophthalmology and
Professor, Department of Ophthalmology
School of Medicine
University of California
San Francisco, California

JONATHAN WIRTSCHAFTER, M.D.
Professor of Ophthalmology, Neurology, and Neurosurgery
Medical School
University of Minnesota
Minneapolis, Minnesota

NEI Program Staff

ISRAEL A. GOLDBERG, Ph.D.
Chief, Retinal and Choroidal Diseases Branch
National Eye Institute
National Institutes of Health

BETTIE J. GRAHAM, Ph.D.
Program Director, Retinal-Vascular Disorders
National Eye Institute
National Institutes of Health

JACK McLAUGHLIN, Ph.D.
Program Director, Retinal Degenerative Disorders
National Eye Institute
National Institutes of Health

PETER DUDLEY, Ph.D.
Program Director, Fundamental Retinal Processes
National Eye Institute
National Institutes of Health

Consultants

Thomas M. Aaberg, M.D.
Milwaukee, Wisconsin

Albert L. Ackerman, M.D.
New York, New York

Anthony Adams, O.D., Ph.D.
Berkeley, California

Alice J. Adler, Ph.D.
Boston, Massachusetts

Ruben Adler, M.D.
La Jolla, California

Bernard W. Agranoff, M.D., Ph.D.
Ann Arbor, Michigan

Gustavo D. Aguirre, V.M.D.
Philadelphia, Pennsylvania

Matthew Alpern, Ph.D.
Ann Arbor, Michigan

Robert E. Anderson, M.D., Ph.D.
Houston, Texas

William H. Annesley, Jr., M.D.
Philadelphia, Pennsylvania

James W. Augsberger, M.D.
Philadelphia, Pennsylvania

Denis A. Baylor, M.D.
Stanford, California

William E. Benson, M.D.
Philadelphia, Pennsylvania

M. Deric Bownds, Ph.D.
Madison, Wisconsin

Michael J. Bradbury, M.D.
Worcester, Massachusetts

George H. Bresnick, M.D.
Madison, Wisconsin

C. David Bridges, Ph.D.
Houston, Texas

Helmut Buettner, M.D.
Rochester, Minnesota

Richard P. Bunge, M.D.
St. Louis, Missouri

Sheldon M. Buzney, M.D.
Boston, Massachusetts

Ronald E. Carr, M.D.
New York, New York

Gerald J. Chader, Ph.D.
Bethesda, Maryland

Bireswar Chakrabarti, Ph.D.
Boston, Massachusetts

Steven T. Charles, M.D.
Memphis, Tennessee

Adolph I. Cohen, Ph.D.
St. Louis, Missouri

Jose G. Cunha-Vaz, M.D., Ph.D.
Chicago, Illinois

Matthew D. Davis, M.D.
Madison, Wisconsin

Francois C. Delori, Ph.D.
Boston, Massachusetts

James G. Diamond, M.D.
New Orleans, Louisiana

J. Graham Dobbie, M.D.
Chicago, Illinois

Edward A. Dratz, Ph.D.
Santa Cruz, California

Thomas D. Duane, M.D., Ph.D.
Philadelphia, Pennsylvania

Ross B. Edwards, Ph.D.
Boston, Massachusetts

J. Terry Ernest, M.D., Ph.D.
Chicago, Illinois

Jay L. Federman, M.D.
Philadelphia, Pennsylvania

Lynette Feeney-Burns, Ph.D.
Columbia, Missouri

Gilbert T. Feke, Ph.D.
Boston, Massachusetts

Carl L. Fetkenhour, M.D.
Chicago, Illinois

Stuart L. Fine, M.D.
Baltimore, Maryland

Daniel Finkelstein, M.D.
Baltimore, Maryland

Constance R. Fitzgerald, M.D.
Gainesville, Florida

Richard Forster, M.D.
Miami, Florida

H. MacKenzie Freeman, M.D.
Boston, Massachusetts

Wayne E. Fung, M.D.
San Francisco, California

J. Donald M. Gass, M.D.
Miami, Florida

Ronald E. Gibson, M.D.
Minneapolis, Minnesota

Bert M. Glaser, M.D.
Baltimore, Maryland

Morton F. Goldberg, M.D.
Chicago, Illinois

Bernice Grafstein, Ph.D.
New York, New York

W. Richard Green, M.D.
Baltimore, Maryland

William T. Ham, Jr., Ph.D.
Richmond, Virginia

Sohan Singh Hayreh, M.D., Ph.D.
Iowa City, Iowa

Paul Henkind, M.D., Ph.D.
Bronx, New York

Tatsuo Hirose, M.D.
Boston, Massachusetts

Joe G. Hollyfield, Ph.D.
Houston, Texas

Kenneth R. Hovland, M.D.
Denver, Colorado

Erwin Hultsch, M.D.
Boston, Massachusetts

William L. Hutton, M.D.
Dallas, Texas

Lee M. Jampol, M.D.
Chicago, Illinois

William H. Jarrett, II, M.D.
Atlanta, Georgia

Eva M. Kohner, M.D., F.R.C.P.
London, England

Juan I. Korenbrot, Ph.D.
San Francisco, California

Theodore Krupin, M.D.
St. Louis, Missouri

Maurice B. Landers, M.D.
Durham, North Carolina

Alan M. Laties, M.D.
Philadelphia, Pennsylvania

Matthew M. LaVail, Ph.D.
San Francisco, California

Mary Lou Lewis, M.D.
Miami, Florida

Richard N. Lolley, Ph.D.
Los Angeles, California

Robert Machemer, M.D.
Durham, North Carolina

Martin A. Mainster, M.D., Ph.D.
Boston, Massachusetts

Michael F. Marmor, M.D.
Palo Alto, California

Richard A. Mathies, Ph.D.
Berkeley, California

Donald E. McMillan, M.D.
Santa Barbara, California

Irvine G. McQuarrie, M.D., Ph.D.
Cleveland, Ohio

Ronald G. Michels, M.D.
Baltimore, Maryland

Sheldon S. Miller, Ph.D.
Berkeley, California

Werner K. Noell, M.D.
Kansas City, Kansas

Robert Nussenblatt, M.D.
Bethesda, Maryland

David S. Papermaster, M.D.
New Haven, Connecticut

Arnall Patz, M.D.
Baltimore, Maryland

Gholam A. Peyman, M.D.
Chicago, Illinois

L. Harrell Pierce, M.D.
Baltimore, Maryland

Ananda S. Prasad, M.D., Ph.D.
Detroit, Michigan

Ronald C. Pruett, M.D.
Boston, Massachusetts

Carmen A. Puliafito, M.D.
Boston, Massachusetts

Lawrence I. Rand, M.D.
Boston, Massachusetts

Melvin L. Rubin, M.D.
Gainesville, Florida

Edward A. Ryan, M.D.
Boston, Massachusetts

Stephen J. Ryan, Jr., M.D.
Los Angeles, California

Felix N. Sabates, M.D.
Kansas City, Missouri

Michael A. Sandberg, Ph.D.
Boston, Massachusetts

Lov K. Sarin, M.D.
Philadelphia, Pennsylvania

Charles Schepens, M.D.
Boston, Massachusetts

Theodore F. Schlaegel, Jr., M.D.
Indianapolis, Indiana

Susan Y. Schmidt, Ph.D.
Boston, Massachusetts

Ariah Schwartz, M.D.
Burlingame, California

Donald M. Shafer, M.D.
New York, New York

Michael Shea, M.D.
Toronto, Ontario
Canada

Jerry A. Shields, M.D.
Philadelphia, Pennsylvania

Irwin Siegel, Ph.D.
New York, New York

Ronald E. Smith, M.D.
Los Angeles, California

Harry G. Sperling, Ph.D.
Houston, Texas

Manfred Spitznas, M.D.
Los Angeles, California

Roy H. Steinberg, M.D., Ph.D.
San Francisco, California

R. Bruce Szamier, Ph.D.
Boston, Massachusetts

Felipe I. Tolentino, M.D.
Boston, Massachusetts

Robert C. Watzke, M.D.
Iowa City, Iowa

Thomas A. Weingeist, M.D.
Iowa City, Iowa

John J. Weiter, M.D., Ph.D.
Boston, Massachusetts

Robert B. Welch, M.D.
Baltimore, Maryland

Frank S. Werblin, Ph.D.
Berkeley, California

Charles P. Wilkinson, M.D.
Oklahoma City, Oklahoma

Paul Witkovsky, Ph.D.
New York, New YorK

James A. Wrath, M.D.
Detroit, Michigan

Herbert Yellin, Ph.D.
Bethesda, Maryland

Richard W. Young, Ph.D.
Los Angeles, California

Keith M. Zinn, M.D.
New York, New York

SUMMARY

INTRODUCTION

THE RETINA IS the delicate, multilayered, light-sensitive membrane that lines the inside of the back of the eye (Figure). Contained within this stratified tissue are a mosaic of photoreceptor cells (the rods and cones) and an exquisitely organized system of other nerve cells and associated elements that contribute to its normal function. Images of the external world that are formed on the retina by the eye's optical elements are converted to electrical signals, encoded into a visual message, and transmitted to the brain via the optic nerve.

The retinal neurons are delicate, highly differentiated cells which, if irreparably damaged, are incapable of being replaced by cellular division. Moreover, they depend for their survival on a carefully controlled environment and a continuous supply of oxygen and nutrients derived from two systems of blood vessels, one intrinsic to the retina, the other in the highly vascular choroid. Any damage to the retina, interruption in its blood supply, or injury to the tissues with which it interacts leads to loss of vision. Unfortunately, the retina is susceptible to injury in a remarkable variety of ways, ranging from hereditary degenerative disorders to diseases associated with infections, and from damage by toxic agents to visual loss resulting from retinal detachment, diabetes, and circulatory failure. Each may have devastating consequences for vision and the conduct of a normal, productive life.

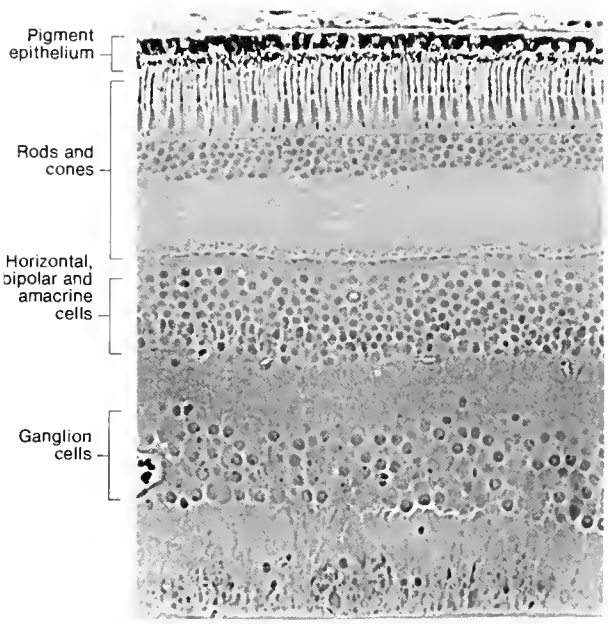


FIGURE. Section through a human retina, showing the principal cell types and layers. Light passes through the entire retina before reaching the outer segments of the photoreceptors, where it is absorbed by the visual pigments and converted into electrochemical signals. Complex processes modify the signals as they are conveyed to the photoreceptors, synaptic terminals, where the visual message is transmitted to adjacent bipolar and horizontal cells for further processing. Additional information processing occurs by means of the complex synaptic interactions of the various retinal cells with one another. The results of this processing appear ultimately in the message carried by the ganglion cell axons (the optic nerve) to the brain. (From Boycott and Dowling, 1969.)

Although the total number of persons afflicted with retinal and choroidal diseases is not known, in 1972 an estimated 1,800,000 Americans had trouble seeing even with glasses because of retinal and choroidal diseases. Within this group, an estimated 514,000 people had so severely impaired vision they could not read ordinary newsprint. Of these, an estimated 200,000 were legally blind, making retinal

and choroidal diseases as a group the leading cause of blindness in the United States. Each year, an estimated 19,000 Americans become blind from these disorders.

In 1972, the latest year in which there are adequate estimates of the economic impact of visual disorders in the United States, retinal and choroidal diseases are estimated to have cost Americans more than $1.5 billion in direct health care expenses and an additional $500 million in lost earnings. Until recently, neither means of cure nor prevention were available for most diseases of the retina and choroid. However, intensive research over the last several years has increased our knowledge of these conditions and in some instances has led to dramatic improvements in diagnosis, treatment, and restoration of vision. Unfortunately, many of the diseases in this group are still poorly understood, and, because of the relative inaccessibility of the retina and choroid, even accurate diagnosis presents a serious problem. The extent and seriousness of this category of ocular disease can best be appreciated by considering the range of pathological conditions to which the retinal and choroidal tissues are vulnerable.

- Diseases affecting the retinal circulation are among the major causes of visual disability and blindness. Diabetic retinopathy, a frequent complication in individuals with diabetes mellitus, is estimated to be the fifth leading cause of blindness in the United States and the third leading cause of new blindness; an estimated 32,600 new cases were present in 1978. Because the retinal manifestations of diabetes often occur in later life, diabetic retinopathy is the second leading cause of blindness among individuals 45–74 years of age. Other retinal vascular disorders, responsible for more than 5,000 new cases of visual impairment each year, include retrolental fibroplasia, sickle cell retinopathy, retinal vein occlusion, and hypertensive and atherosclerotic vascular disease.
- Developmental and hereditary disorders of the retina and choroid are responsible for 20 percent of all legal blindness due to chorioretinal diseases. Retinitis pigmentosa alone accounts for nearly 1,500 new cases of legal blindness each year; as many as 100,000 people may be afflicted with the disease in this country. Like most of the disorders in this category, retinitis pigmentosa strikes the young, causing a lifetime of hardships for them, and heavy financial and emotional burdens for their families.
- Macular degeneration and other disorders that affect the central retinal area subserving acute vision lead to severe visual impairment. According to 1970–1974 data from the United States, an estimated 165,000 first visits to physicians were made each year because of macular degeneration, making it one of the Nation's major public health problems.
- In 1970–1974, because of retinal detachment and vitreous disorders that lead to a separation of the neural retina from the underlying pigment epithelium more than 25,000 first visits to physicians were made each year. Although the retina can be surgically reattached in many instances, approximately 7,000 of these cases suffer irreparable damage and extensive loss of vision. In addition, separation of the vitreous from its normal attachment sites is an important factor in diabetic retinopathy, contributing to the abnormal blood vessel growth and subsequent vitreous hemorrhage that characterize this blinding disease.
- Although less frequent, inflammatory disorders of the retina and choroid comprise a large group of destructive, often painful diseases. Characterized by the accumulation of inflammatory cells and fluid in the ocular tissue, these diseases often affect not only the retina and choroid but also the vitreous body and the front of the uvea (the ciliary body and iris); involvement of the latter tissues may result in secondary glaucoma and cataract. In 1972, approximately 23,000 cases of legal blindness in the United States were attributable to uveitis, and an estimated $10 million was spent in medical care for these patients.
- Tumors of the retina and choroid, principally choroidal melanoma and retinoblastoma, have a relatively low incidence, but their importance is magnified by the fact that these cancers can cause death as well as blindness. About 1,500 new cases of choroidal melanoma are diagnosed annually in the United States, with an overall mortality at five years of 35 to 90 percent, depending on the size and degree of malignancy of the tumor. Retinoblastoma is probably the most common of all congenital tumors affecting the newborn. It appears to occur more frequently among black children, and although the tumor is responsive to various forms of treatment, the visual loss it causes is usually severe and permanent.
- Toxic or environmental agents, acting separately or in concert, can severely damage the retina, even though they may appear harmless to other tissues. In addition, chemotherapeutic agents introduced into the eye or bloodstream may affect adversely the retina and optic nerve with serious consequences for visual function.
- The photoreceptors are particularly susceptible to injury and are affected by a wide variety of choroidal and retinal diseases. They are often the first cells to degenerate or suffer damage from hereditary defects (such as retinitis pigmentosa),

retinal detachment, nutritional deficiencies, circulatory disturbances, overexposure to light, and the toxic effects of drugs. It is essential that we understand all aspects of the normal functional properties of the visual cells and the unique ways in which specific disease processes alter their metabolic and structural integrity.

- Although not an integral component of the visual sensory pathway, the retinal pigment epithelium is nevertheless a central element in the health and survival of the visual cells. Among its many functions are the selective transport of metabolites to and from the photoreceptors and the daily removal of discarded photoreceptor debris. Failure to perform any of its functions adequately results in death of the neighboring receptors and blindness within the affected area. In addition, the pigment epithelial cells may help keep the sensory retina in its proper position, a potentially significant factor in the etiology of retinal detachment.
- An understanding of retinal organization, the chemical substances that transmit information between retinal cells, and the molecular events that underlie visual adaptation is needed for a true understanding of the visual process and for the prevention, diagnosis, and treatment of retinal and choroidal diseases. It is also important to recognize that the retina is part of the brain and, as in a number of brain disorders, abnormalities in retinal function may result from glial cell pathology or disturbances in the manufacture or utilization of essential neurotransmitter agents.

Significant advances have been made in recent years in understanding most of these conditions, with concomitant improvements in the methods by which they are treated medically and surgically. In the case of inflammatory disorders, for example, considerable progress has been made in identifying and isolating infectious agents that cause uveitis, and recently developed fluorometric techniques have greatly facilitated the ability to diagnose the vascular lesions that accompany inflammatory reactions of the retina and choroid. With regard to intraocular tumors, better diagnostic procedures have led to more accurate identification of the tumor type, and methods of treatment, particularly for childhood tumors such as retinoblastoma, are now much improved.

Research has led to a deeper appreciation of the role of the blood-retinal barrier in the normal functioning of the retina. The breakdown of the barrier that occurs in diabetic retinopathy and other vascular diseases enables water and other molecules to leak into the retinal substance, resulting in macular edema and severe visual loss. Investigators also have a better understanding of the pathogenesis of macular disease and improved methods for identifying subtle clinical changes as they develop. A major advance has recently been made in the ability to treat the neovascular type of aging-related maculopathy, but management of many of these conditions remains disappointing.

Knowledge of hereditary and developmental disorders has increased substantially over the last five years, largely because a great deal has been learned about the structure, function, and biochemistry of the retina and pigment epithelium. In some cases, enough information has been obtained to begin therapeutic trials. The discovery of animals with hereditary disorders and the identification of specific biochemical defects in their photoreceptors provides a basis for considering similar pathogenetic mechanisms in human disease. More sophisticated tests now permit detection of carriers of hereditary chorioretinal disorders in certain instances, for example, in such conditions as vitelliform macular degeneration and choroideremia.

In retinal detachment and vitreous disorders the major advances have been in the form of new instrumentation and better methods of surgical management. The development and application of vitrectomy surgical techniques, in particular, has been an outstanding achievement. Retinal detachment surgery now has a success rate of 80 to 90 percent, resulting in a significant restoration of visual function.

The research on the conditions outlined above is applicable to other ocular and systemic disorders, particularly those related to the vascular problems found in diabetes and sickle cell disease. For example, the information gleaned from studies of the retinal vasculature in diabetes may have importance to kidney function in this disease, and there is obvious overlap in the relationship of retinal vascular occlusions to systemic hypertension and carotid insufficiency.

The fact that the retina is an outgrowth of the brain and that the optic nerve is homologous with neural tracts in the spinal cord provides a commonality of interest among investigators of nerve function in the retina and in the central nervous system. The pressing problems associated with information processing, neuronal regeneration, cell death, aging, neuron-glia interactions, nerve growth factors. and a host of related topics are a shared concern, and the research findings in these fields are of mutual importance.

PROGRAM STRUCTURE

In its deliberations, the Panel distinguished between research on problem areas and specific disorders and research on development, structure, and func-

tion as related to disease. In addition, cross-cutting areas of interest that are in need of research support were identified. The 14 subprograms examined by the Panel were:

Vascular, Inflammatory, and Neoplastic Disorders of the Retina and Choroid

1. Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities
2. Inflammatory Disorders
3. Tumors

Degenerative Disorders of the Retina

4. Developmental and Hereditary Disorders
5. Macular Degeneration
6. Retinal Detachment and Vitreous Disorders
7. Toxic and Environmental Disorders

Fundamental Processes and Retinal Disorders

8. Retinal Pigment Epithelium
9. Photoreceptors, Visual Pigments, and Phototransduction
10. Retinal Organization, Neurotransmission, and Adaptation
11. Glial Cells and the Retinal Microenvironment
12. Rescue and Regeneration of Neurons in the Optic Nerve and Retina

Related Areas of Research Opportunity and Need

13. Noninvasive Techniques in the Study of Retinal Disorders
14. Tissue Acquisition and Distribution: Human Donor Eyes and Animal Models

In considering these various categories and classifications, it is apparent that the problems raised by individual conditions are often interrelated, and hence, research areas are interdependent. One problem area often merges imperceptibly into others, and the efforts of investigators from several disciplines may be required for their solution.

Thus, the classification of retinal and choroidal disorders and research areas is essentially pragmatic; in the main, it lists the chief clinical problems and enumerates individual ocular regions and disciplines. Overall, this classification reflects the best current approach to the solution of clinical problems: to apply what is now known in an attempt to lessen the ravages of disease while pursuing, through the intelligent development and application of basic science, means of prevention and methods of cure.

The National Eye Institute's research program in Retinal and Choroidal Diseases encompasses specific clinical disorders and basic research on the development, structure, and function of the eye as related to disease. The opportunity to diagnose, treat, and ultimately prevent disease depends in large measure upon fundamental research. Often disorders seen in the clinic help to focus research on abnormal processes, but without a full basic understanding of the normal condition, it is virtually impossible to comprehend what is wrong in the abnormal state or to find the means to correct it. To gain best advantage toward the goals set forth below, the Panel strongly urges that adequate sustained, coordinated support be given to both basic and clinical research.

ORGANIZATION OF THE PLAN

Each chapter in this report begins with an introduction that highlights the importance of the research field or disorder it addresses. This is followed by a list of subprogram objectives, an overview of current research support, a review of recent research accomplishments, and a discussion of current research needs and opportunities. This analysis culminates in a list of the Panel's recommendations for the Program Base and for Program Development Priorities within the subprogram.

The Program Base includes areas of ongoing research where the current level of activity is considered adequate, or areas of ongoing research in which there may be great need for additional activity, but where, in the Panel's judgment, little or no opportunity (new methods or insights) exists at present to justify a significant expansion of effort. Nonetheless, additional applications for research grants in these areas may be funded if they are innovative and of very high quality as determined by the NIH peer review system.

Program Development Priorities include areas of ongoing research in which new knowledge and techniques offer particular opportunities for scientific progress, or promising new areas of research in which there is little or no support at present but where there is both great need and high potential for success. Such areas are judged to warrant significantly increased support over the next five years, provided that high quality applications for research grants in these areas are forthcoming.

Each chapter concludes with a table that shows the number and dollar amount of research grants supported in each of these areas in FY 1981 and the number and estimated costs of projects the Panel recommends for funding by FY 1983. For a detailed discussion of the planning process used to develop these recommendations see *Volume One, The 1983 Report of the National Advisory Eye Council.*

PROGRAM GOALS

Recognizing the research opportunities that lie ahead and the magnitude of the disease problems to be faced, the Retinal and Choroidal Diseases Panel has set the following broad program goals to guide research in this area:

- To increase basic knowledge of the development, metabolism, molecular structure, and functional properties of the retinal neurons and the glial, choroidal, and pigment epithelial cells upon which they depend for maintenance and proper function.
- To develop procedures for the prevention and cure of diabetic retinopathy, retinal degeneration, retinal detachment, and other chorioretinal disorders, and conduct controlled clinical trials of promising new therapeutic measures as they become available.
- To devise noninvasive methods for probing the functional state of the human retina and its neighboring tissues, as an aid in diagnosis and in identifying the cellular elements involved in disease processes.
- To investigate in animals affected with retinal and choroidal diseases the genetic, biochemical, and immunological bases of the pathological processes, and the effectiveness of innovative therapeutic approaches.
- To identify nutritional and environmental factors that may be toxic to the retina, interfere with its normal development, or affect the long-term survival of the visual cells.
- To improve methods for maintaining retinal and choroidal cells in tissue culture, for investigating normal and pathological cellular mechanisms, analyzing the molecular basis of neurotransmission, testing the efficacy of new forms of therapy, and discovering the factors that promote neuronal growth and regeneration.
- To establish a sound basis for prognosis, genetic counseling, and medical intervention by determining the etiology, natural history, and epidemiology of inflammatory disorders, tumors, and the various degenerative diseases affecting the retina and choroid.

OVERVIEW OF CURRENT RESEARCH SUPPORT

In FY 1981, the National Eye Institute funded 381 research grants, at a total cost of $33,535,000, in the Retinal and Choroidal Diseases program. Of these, 53 grants were for research on diabetic retinopathy and other circulatory abnormalities of the retina and choroid. In addition, the NEI currently has contracts with 17 clinical centers for the Diabetic Retinopathy Vitrectomy Study, 23 clinical centers for the Early Treatment Diabetic Retinopathy Study, and 5 additional centers for ancillary functions related to these programs. NEI funded 14 research projects dealing with inflammatory disorders of the retina and choroid in FY 1981.

With regard to ocular tumors, 18 research projects were funded by the National Eye Institute in FY 1981 for studies of ocular melanoma and retinoblastoma. Studies of the diagnosis, etiology, pathogenesis, and prognosis in these diseases include such areas of investigation as immunodiagnosis, correlation of malignant potential with histopathological findings, experimental chemotherapy, and biochemical and cytogenetic markers in animal models and cultured cell lines.

In the field of developmental and hereditary disorders, 42 National Eye Institute grants were supported in FY 1981. This support has permitted electrophysiological and psychophysical testing, fundus reflectometry measurements, and, where possible, a correlation of the results of these test procedures with ultrastructural and biochemical studies of animal models and postmortem donor eyes from patients with these diseases.

In FY 1981, the National Eye Institute supported 21 grants for research on macular degeneration, among which is a collaborative therapeutic trial, the Macular Photocoagulation Study. The Institute funded 16 research grants in FY 1981 dealing with retinal detachment and vitreous disorders. Almost one-half of these involved basic studies of the development, structure, and function of the vitreous; the junction between retina and vitreous; and the factors that normally maintain retinal attachment. The clinically oriented grants dealt primarily with improvements in instrumentation and techniques for vitrectomy and retinal detachment surgery, management of vitreous trauma, and studies of vitreoretinal membrane shrinkage.

Toxic and environmental hazards are the concern of numerous government agencies, private foundations, and various segments of industry. Although several projects relating to ocular toxicity were funded by the National Eye Institute in FY 1981, only two were directly concerned with the effects of potentially toxic agents on the choroid and retina.

These dealt with the toxicity of intravenously administered fluorescent dyes used in angiography, the toxic effects of polycyclic aromatic compounds such as chloroquine, and the retinotoxic effects of antibiotics used in treating ocular diseases and administered intravitreally for the treatment of endophthalmitis.

The National Eye Institute supported 26 grants in FY 1981 devoted to the study of the retinal pigment epithelium and its interactions with the visual cells. Most of these dealt with renewal phenomena; others were concerned with questions of transport, cell biology, biochemistry, physiology, or development. In the field of photoreceptor physiology, 112 grants were funded; about half of which dealt with transduction and the normal functional properties of the visual cells, others with photochemical reactions and the structure of rhodopsin and the disk membranes, and the remainder primarily with metabolic processes in visual receptors.

In FY 1981, 57 grants for research on retinal organization, neurotransmission, and adaptation were supported by the National Eye Institute. In the main, the fields of study included growth and development of retinal cells, structural and electrophysiological analysis of neuronal interactions, identification of the neurotransmitter agents at synaptic sites, the retinal mechanisms subserving color vision and adaptation, and the molecular events involved in the generation of electrical signals by the retinal nerve cells.

Although six National Eye Institute research grants dealt with glial cells and the retinal environment in FY 1981, a number of research projects in retinal metabolism and cellular physiology encompassed this area of interest. Moreover, glial cells play a vital role in neuronal injury and the problems associated with nerve regeneration; thus, projects relating to glial function complement two supported studies of retinal neuronal regeneration.

The development of methods for the noninvasive study of retinal physiology was the focus of 12 National Eye Institute research grants in FY 1981; in addition, noninvasive techniques were developed and used extensively in many of the clinical projects mentioned previously.

A program to supply Irish setters afflicted with rod-cone dysplasia and miniature French poodles with progressive retinal atrophy was in operation in FY 1981. Numerous research centers now utilize animal models for studies on the etiology of retinal degeneration, and individual laboratories have, with the cooperation of regional eye banks, obtained postmortem human donor eyes for ultrastructural and biochemical studies of chorioretinal diseases.

The foregoing indicates that the National Eye Institute's program to curb visual disability from chorioretinal diseases represents a broad-based system of support that attempts to encompass the various aspects of normal function and almost every serious pathological condition. Support is also provided by other components of the National Institutes of Health and other agencies of the Department of Health and Human Services, the National Science Foundation, Department of Defense, Veterans Administration, and other Federal organizations. Significant funding was also provided by private national philanthropic organizations, most notably the National Retinitis Pigmentosa Foundation, Fight for Sight, Inc., and Research to Prevent Blindness, Inc. For a complete list of projects supported by the NEI in FY 1981 and other organizations in 1980, see *Volume Three, Support for Vision Research*. Nevertheless, it will become apparent in the description of research needs and subprogram priorities that a greater effort in some major areas of concern and a reapportionment of effort in other areas is desirable to take advantage of new opportunities that have emerged from past research.

RECENT ACCOMPLISHMENTS

Considerable progress has been made in recent years toward realizing some of the major goals of the National Eye Institute's Retinal and Choroidal Diseases program. A multitude of technical innovations, and the introduction of powerful analytical procedures culled from the various scientific disciplines, have spurred advances on all fronts. Most notable have been an impressive increase in knowledge of normal retinal function, better understanding of disease processes, and improvements in diagnostic and therapeutic procedures that have led to prevention of blindness and restoration of useful vision in patients with serious chorioretinal disorders.

Progress in elucidating the mechanisms of visual excitation and adaptation, which is central to a complete understanding of the visual process, has been remarkably rapid. Novel techniques for analyzing the currents that flow across photoreceptors are being used to identify the molecular events that link visual pigment activation and the cell's electrical response, and specially constructed ion-selective electrodes are probing the ionic basis of the excitatory process. Sensitive biochemical assays have made possible the detection of light-induced enzymatic changes that are vital to normal cellular function, and the identification of various transmitter agents used by the retinal neurons for intercellular communication. The development and synaptic connections of the retinal neurons are being described in ever-increasing detail with new methods

for tracing the course of nerve fibers and visualizing the molecular detail of membrane surfaces. Elegant studies that combine electrophysiological and histochemical techniques have revealed much about the functional significance of the retina's cellular stratification. In addition, a growing awareness of the susceptibility of the visual and other retinal cells to damage by light, environmental pollutants, pesticides, and other toxic agents has led to new concepts that may have important consequences for the preservation of sight.

A number of important facts have been established about the intimate relationship between the sensory retina and the layer of pigment epithelial cells, and it is now more apparent than ever that an upset in the functional integrity of the pigment epithelium can cause widespread destruction of the retina. The discovery of specific retinol-binding proteins for the transport of vitamin A isomers between the photoreceptors and pigment epithelial cells has greatly enhanced understanding of the rhodopsin cycle. Moreover, intercellular junctions between the pigment epithelial cells form a part of the blood-ocular barrier that regulates the flow of molecules to and from the visual cells; a breakdown of this barrier occurs in inflammatory disease, diabetic retinopathy, and some of the retinal dystrophies.

Striking advances have been made in the medical and surgical management of chorioretinal diseases. Autoimmune diseases of the retina and choroid that once had a hopeless prognosis have yielded to immunosuppressive agents, and severe infectious ocular diseases have been treated satisfactorily with a number of new antimicrobial drugs. Vitrectomy, a procedure for evacuating hemorrhages from the vitreous gel and removing bands of fibrous membranes that can cause retinal detachment, has proved remarkably successful in restoring vision to patients facing blindness from diabetic retinopathy, inflammatory disorders, and trauma.

Proton beam irradiation has been used successfully in the treatment of choroidal melanoma. Ultrasound, employed routinely for detection and localization of foreign bodies and intraocular lesions, has been used to disrupt vitreous membranes and may find application also in tumor therapy.

The nationwide Diabetic Retinopathy Study, sponsored by the NEI, demonstrated that visual disability from the proliferative form of diabetic retinopathy can be sharply reduced by extensive retinal photocoagulation with the xenon arc or the argon laser. Another multicenter NEI-supported clinical trial, the Macular Photocoagulation Study, has recently led to a dramatic breakthrough in the treatment of the neovascular form of aging-related maculopathy (senile macular degeneration).

Improved diagnostic and laboratory procedures have also made a significant impact on progress in this field. Vitreous fluorophotometry, although still in need of improved standardization, is a promising new technique for revealing early vascular changes that precede the overt signs of diabetic retinopathy. While the search for an angiogenesis factor that causes new vessel formation continues, the development of an assay system for the factor using cultured capillary endothelial cells represents a major step forward in this effort. Tumor-specific immune responsiveness has been demonstrated in patients with choroidal melanoma, and the successful induction of uveal melanomas in cats using feline sarcoma virus should provide a valuable model in which to investigate the pathogenesis of this tumor. Genetic and chromosomal studies have contributed to a better understanding of retinoblastoma, and the establishment of in vitro cell lines of retinoblastoma has made a continuous supply of tumor cells available for use by investigators.

Retinal tissues obtained postmortem from human donor eyes and animal models of chorioretinal disease provide one of the most fertile fields of investigation, which has already begun to yield important dividends. For example, new opportunities for studying disciform macular degeneration have become available through the development of a reproducible model of subretinal neovascularization in the monkey, and some of the factors involved in the vascular reaction have already been identified. Specific biochemical and enzymatic defects have been identified in the visual cells of animals with hereditary retinal diseases. Careful ultrastructural studies of retinal cells from donor eyes have revealed the pathological changes that occur at various stages in the degenerative process. Moreover, methods for maintaining pigment epithelial cells in culture have been developed, and the cells from donor eyes of patients with retinitis pigmentosa are being analyzed for disease-specific biochemical markers and chromosomal abnormalities.

Although chorioretinal biopsy may become a practical and relatively safe diagnostic procedure, noninvasive methods remain the principal means of assessing the functional state of the retina. The value of noninvasive approaches is well documented in every field of retinal study, but is perhaps most vividly illustrated in the diagnosis of hereditary retinal degenerations. Electroretinography, for example, has proved invaluable for the diagnosis of retinitis pigmentosa in early life even before the appearance of changes in the fundus, and abnormalities in the electrical response of the retina have been used to detect obligate carriers of the X-linked form of the disease. In addition, the electroretinogram, used in conjunction with fundus reflectometry and a series of psychophysical test procedures, has made possible in some instances the identification of the retinal cell types affected by the disease process, the

differential diagnosis of subclasses of pathology, and a means by which to chart the spread or remission of pathology within and across the retina.

RESEARCH NEEDS AND OPPORTUNITIES

The recent successes of the nationwide Diabetic Retinopathy Study and the Macular Photocoagulation Study illustrate the value of large-scale clinical trials in assessing new treatments for ocular disease. A collaborative effort should be made to determine whether "tight" blood glucose control in diabetes is effective in preventing vascular complications such as retinopathy. In addition, basic studies are needed to further understanding of retinal vascular diseases. For instance, tissue culture of retinal capillary cells should prove useful in learning how these cells become abnormal in diabetic retinopathy and other vascular disorders, and the study of capillary endothelial cells in culture may permit identification of angiogenic factors that contribute to the formation of new vessels on the surface of the retina. Methods to study retinal blood flow require refinement, and improved techniques for measuring blood oxygenation may contribute to a better understanding of the etiology of retinal vascular disease.

The etiology of most inflammatory disorders is still unknown. New efforts should be directed to developing methods for identifying pathogens in ocular tissues. Also, specimens of affected tissues should be examined for immunologic factors, because it is likely that different forms of uveitis can be distinguished on the basis of the immunologic components participating in a given reaction. Moreover, there is a need to analyze genetic factors that may predispose some individuals to specific types of inflammatory disease.

The clinical management of ocular melanoma presents a serious problem. Randomized controlled clinical trials are needed to assess the effectiveness of treatments. It is important to standardize cytologic classification of these tumors and to determine whether such findings can be correlated with prognosis. In addition, an attempt should be made to identify risk factors, such as sex, age, environmental exposure, and race, that may be associated with the incidence of ocular tumors. In the search for new methods of treatment, a human choroidal melanoma cell line should be established for biochemical, immunologic, and chemotherapeutic studies. A cell line should be established also for retinoblastoma to determine whether the presence of vitamin A receptor sites in the tumor cells can be used to advantage in the eradication of the tumor itself.

Because retinal and choroidal degenerations are often genetic in origin, there is a need to identify specific chromosomal abnormalities in affected individuals, with the ultimate goal of defining and correcting the biochemical defects in these diseases, detecting carriers, and developing prenatal tests to identify affected offspring. To this end, intensive study of postmortem human donor eyes should be encouraged. Noninvasive tests should be used to follow the natural history of the disease process and to provide a better understanding of the underlying pathophysiologic changes. Basic research is needed on the genetic, antigenic, and environmental factors that cause abnormal blood vessels to develop beneath the retina, and the aging-related biochemical changes in Bruch's membrane, which enable the choroidal vessels to grow inward beneath the macular region. In addition, further investigation of possible therapeutic agents, for example, alpha-adrenergic blockers, for cystoid macular edema should be encouraged. Clinical trials should be continued to determine the long-term results of photocoagulation treatment in the management of aging-related maculopathy (with neovascularization) and to evaluate its usefulness in the management of the presumed ocular histoplasmosis syndrome.

Major advances in the treatment of retinal detachment and vitreous disorders will depend in large measure on basic research into the development, metabolism, permeability, and function of the vitreous gel in normal and disease states. Particular emphasis should be placed on studies dealing with the factors that induce membrane formation on the surface of the retina, a major cause of failure in retinal detachment surgery. In addition, clarification of the role of the retinal pigment epithelium in producing adhesive forces that keep the retina in place may provide new insights into the causes of retinal detachment.

Other aspects of pigment epithelial cell function are equally important in retinal and choroidal diseases. A great deal is already known about the cyclic nature of the receptor outer segment renewal mechanism and the role of the retinal pigment epithelium in phagocytizing the shed discs. Nevertheless, important questions remain concerning the changes that occur in the "older" portions of the outer segment, the trigger mechanism that initiates shedding, the recognition factor that leads to phagocytic action, and the changes in these events that occur with aging and in retinal disease. The transport properties of the pigment epithelium across the barrier it creates between the choroidal blood supply and the visual cells require further study, and additional research is also needed to determine how this barrier is affected in inflammatory diseases, macular degeneration, and other retinal disorders.

Studies of photoreceptor physiology and the structure and function of rhodopsin are well supported and making excellent progress. However, greater emphasis is needed on research into receptor metabolism, in particular those aspects involving protein transport, the regulatory mechanisms that govern the rates of disc shedding and synthesis, and the interplay of photic exposure and nutritional deficiencies in producing visual cell death.

Although rapid progress has been made toward providing a complete description of the functional organization of the retina, much still has to be learned about the intricate "wiring diagram" for retinal neurons and the chemical messengers that effect neurotransmission at synaptic junctions. A large number of substances have been implicated as retinal neurotransmitters, but more definitive tests are needed to establish which transmitters are used by the various specific types of retinal cells, determine their actions on postsynaptic elements, and locate the enzymatic mechanisms responsible for their synthesis and degradation. It is also essential to define the role of the retinal microenvironment in these events, in the processes affecting visual adaptation, and in the generation of the electroretinogram. Indeed, the cellular origins of the electroretinogram may soon be completely identified, and the results should greatly enhance the usefulness of this valuable diagnostic test.

A concerted effort should be made to develop sensitive, easily applied, noninvasive test procedures directed at problems relating to specific disease entities. Almost every area of clinical investigation requires better noninvasive methods for diagnosing pathological conditions, establishing the nature of the underlying defect, evaluating visual function, following the course of the disease process, and assessing the efficacy of therapeutic measures.

The risk of disease from exposure to toxic and environmental agents that are damaging to the retina needs to be defined through carefully controlled epidemiological studies relating incidence and extent of injury to degree of exposure. Laboratory studies should be initiated and appropriate procedures developed for assessing drug toxicity to the eye, determining the routes by which toxic agents reach the retina and choroid, and localizing by autoradiography the sites at which the various substances act. Moreover, advantage should be taken of recent progress in organ and tissue culture to test the ocular toxicity of new drugs in vitro before their release for public use.

It is essential to take greater advantage of extensive current research on the growth, regeneration, and plasticity of nerve cells in the brain and spinal cord, and explore the possibility of applying the results of such studies to the treatment of retinal and optic nerve injuries. Although there is increasing interest in the pathogenetic mechanisms underlying retinal degenerations, little attention has been paid to factors that might promote the survival of nerve cells and prevent the harmful proliferation of glial elements in the retina. At the cellular level, the initiation and control of axoplasmic transport needs to be defined further, and the relationships between events occurring at the cut end of an axon and the cell body need to be determined to find ways to enhance axonal regrowth.

The acquisition and distribution of human donor eyes and animal models with inbred or acquired ocular pathology represents another broad-based research need that applies to a variety of diseases involving the retina and choroid. Programs for obtaining human donor eyes as soon as possible after death should be encouraged, and the special protocols to be followed for specific needs should be well defined and distributed to all participants. The development of new animal models of the various disease categories is urgently needed, as well as adequate facilities to breed these animals for interested investigators.

TRAINING AND MANPOWER NEEDS

The realization of the above research goals and fulfillment of the foregoing needs will depend on the availability of a dedicated cadre of well-trained, innovative investigators. It is hoped that, spurred by this report and by the astonishing pace of technological advance, a reorientation of many established vision scientists toward new areas of opportunity will occur. But this will not be enough. To a significant extent, real progress will depend on the attraction to vision research of new investigators with special skills. Opportunities have been highlighted in the Plan for scientists trained in the basic research areas—neuroscience, molecular biology, pharmacology, physiology, biomedical engineering, optics, psychophysics, and immunology—to apply their talents and skills to problems in vision research. There is also a great need for the clinician investigator who is uniquely trained to recognize opportunities for research on human diseases and to formulate, design, and carry out projects to capitalize upon them. Increasingly, clinically relevant questions can be asked at the cellular and molecular level; thus, the need for the interaction of well-trained basic science and clinician investigators and the pooling of their diverse talents is clear.

Special attention should be placed on training investigators in the following areas as they relate to research on the disorders of the retina and choroid:

- Genetics and molecular biology;
- Immunology;
- Nutrition and metabolism;
- Pharmacology and toxicology;
- Control of cellular proliferation;
- Epidemiology and biostatistics;
- Neuroscience (retinal development, regeneration, and plasticity); and
- Noninvasive tests of retinal function.

SUMMARY OF 1983–1987 RECOMMENDED PROGRAM DEVELOPMENT PRIORITIES

The Panel's specific recommendations for priority areas within each subprogram are included in the respective chapters of this volume. These recommendations should serve as a guide and are not intended to stifle innovative projects that offer promise of important breakthroughs for the prevention and cure of chorioretinal diseases. They are based on an assessment by the Panel members and their consultants of the current state of knowledge of the areas most likely to yield significant information relating to the goals of the overall program. In summary, the Panel recommends that:

- Fundamental research studies continue to be pursued vigorously in the following areas:
  - The structure, biochemistry, metabolism, and physiology of the visual cells and pigment epithelium with special emphasis on their functional interactions and regional differences throughout the retina.
  - The development and functional organization of the retina and the identification of its synaptic mediators and adaptive mechanisms.
  - The characteristics of the normal retinal and choroidal circulation and the etiology and pathogenesis of the changes that occur in diabetic retinopathy and other vascular disorders.
  - The composition, metabolism, and permeability of the vitreous gel, and the changes that occur with new vessel formation, vitreous banding, and retinal detachment.
  - The nature and origin of ocular neoplasms.
  - The genetic and biochemical changes associated with inherited retinal disorders and other chorioretinal diseases.
  - The use and further development of noninvasive methods for the study of retinal and choroidal function in normal and in pathological conditions.
  - Identification, development, and maintenance of appropriate animal models of the various acquired, developmental, and hereditary retinal and choroidal diseases.
- Special attention be given to the following neglected areas of research:
  - Macular degeneration—especially with regard to aging-related changes in Bruch's membrane, the formation of abnormal new blood vessels, and the metabolism of the pigment epithelium-photoreceptor complex within the macular area.
  - The cellular mechanisms that contribute to the adhesive forces between the retina and pigment epithelium and the factors that may contribute to retinal detachment.
  - The role of the blood-retinal barrier in macular degeneration, retinal detachment, vasoproliferative retinopathies, and ocular toxicity to drugs.
  - The role of the immune system in the development of inflammatory disease, retinal degeneration, and ocular tumors.
  - The identification of infectious agents and the determination of the role of autoantigens in the induction of inflammatory disease.
  - Nerve-glial cell interaction in relation to the functional and regenerative properties of the retinal neurons, and the origins of the electroretinogram.
  - The development of tissue culture methods for the study of ocular drug toxicity, ocular tumors, and degenerative diseases of the retina.
  - Retinal disorders resulting from toxic, environmental, and nutritional factors.
  - Methods to prolong retinal survival and the factors that contribute to rescue and regeneration of nerve cells in the retina and optic nerve.
  - The effects of photic exposure and the aging process on the visual and pigment epithelial cells.

- Controlled clinical trials be conducted to evaluate the effectiveness of promising new treatments for retinal and choroidal diseases.

IMPLEMENTATION OF THE PLAN

The individual, investigator-initiated, NIH research project grant continues to be the National Eye Institute's predominant and highest priority funding mechanism. Therefore, the successful implementation of the recommendations of the Retinal and Choroidal Diseases Panel, as well as those of the other Panels that have contributed to *Vision Research—A National Plan: 1983-1987,* will depend largely upon investigators submitting grant applications for research in the scientific areas the Panel has identified for emphasis. Because scientific merit, as evaluated by the traditional NIH peer review system, will continue to be the principal determinant of which approved grant proposals the NEI will fund, those approved applications having the best "priority" scores assigned by NIH initial review groups will be funded. Applications with mid-range scores will be paid as funds are available; however, some may be specifically designated by the Council as having "High Program Relevance" (that is, fulfilling one of the Plan's recommendations, especially in an area of research considered to be underfunded), and are recommended for placement in a more favorable position for funding. Applications with poorer scores will not be funded—even if they propose research on a topic the Panel has judged to be in need of additional or new support (Chart).

By using such a system, NEI supports scientific excellence, innovation, and creativity while carrying out its mission of supporting research aimed at alleviating blindness and visual disability. The Na-

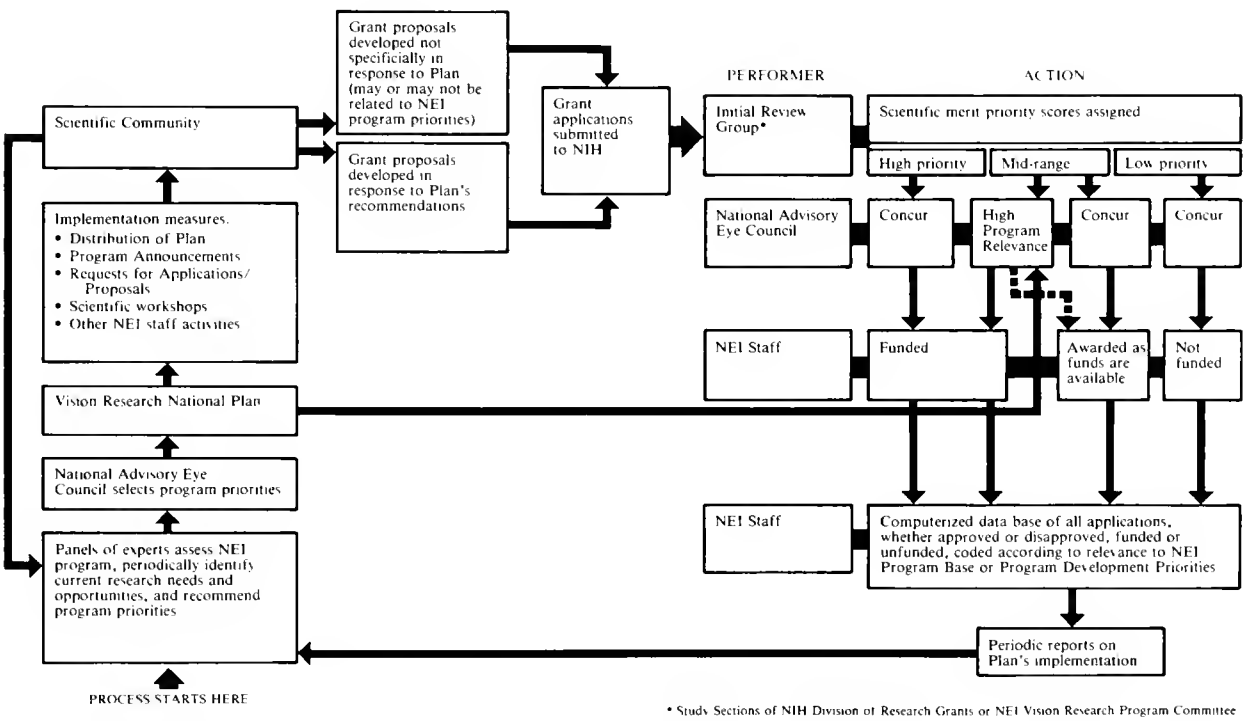


CHART. National Eye Institute Program Planning System. Both the NIH scientific merit priority scores and the program priorities established in the National Plan help determine which grant applications NEI will fund. All applications with high scientific merit priority scores are funded regardless of their relevance to program priorities. Some applications with mid-range scores and judged to be highly relevant to program priorities are singled out and placed in a better funding position than they would have been on the basis of the score alone.

tional Advisory Eye Council will monitor the responses of the research community to the recommendations in the Plan as well as new research advances and developing opportunities, and recommend to the NEI staff on a regular basis what further implementation measures or changes in program priorities may be required. For further discussion of the Plan's development and implementation, see *Volume One, The 1983 Report of the National Advisory Eye Council.*

RESOURCE REQUIREMENTS

The following table presents a summary of the Panel's estimates of the number of grants necessary to carry out its recommendations for each of the Retinal and Choroidal Diseases subprograms in FY 1983. The actual number and cost of grants funded in each subprogram in FY 1981 (the base year of the Plan) are shown in the first column. The second column indicates the number of additional (or fewer) grants the Panel believes should be funded in each subprogram through the end of FY 1983, based on an analysis of current research and of future needs and opportunities. The total number of grants for FY 1983 for each subprogram indicated in the third column is the estimated sum of new and continuing awards to be made in that year along with an estimate of their cost.

For example, the first line of the table shows that 51 grants were actually awarded in FY 1981 for Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities. Because about one-third of all NEI grants terminate in any given year, in making its estimates for 1983 the Panel assumed that about 17 of the 51 projects funded in FY 1981 would terminate in that year, thereby making funds available for new or renewal grants in this subprogram in 1982 and that another 17 would terminate in that year. The Panel then judged that an additional 9 grants would be required by 1983 to meet its recommendations in this subprogram. Therefore, of the total of 60 projected awards in this subprogram for FY 1983, approximately 17 would be ongoing and 43 would be new or renewal awards to be funded during FY 1982 and FY 1983. Thus, even in those subprograms for which the Panel projected fewer grants by FY 1983 than were funded in FY 1981, the potential still exists for awarding new research projects in areas indicated for emphasis.

The actual number of grants funded in these areas may of course be either more or less than these projections indicate, depending on the quality, kind, number, and costs of the grant applications NEI receives and the actual availability of funds. Concerning funding, it must be emphasized that the six Panels' dollar estimates for FY 1983 do not necessarily indicate what the actual National Eye Institute extramural research budget will be for that year. However, because the Panels' estimates are based upon detailed documentation of projected research needs and costs, it is hoped that those in the Executive and Legislative branches of the Government who make the final decisions concerning the NEI budget will use them in making informed judgments about the resources required for the support of vision research. In making these estimates the Panels took into account the following factors for each category of research considered:

- Degree of relevance to the program's goals and objectives
- Current level of support by NEI and other organizations
- Recent research accomplishments
- Potential for future development
- Availability of trained manpower
- Likelihood of significant progress over the next three to five years.

The Panel recognizes that in addition to scientific judgments, social, economic, and political considerations will shape the final NEI budget for each year. Therefore, no attempt has been made in this report to make detailed resource estimates beyond FY 1983, although the Council has projected an overall budget for the NEI through FY 1985 (*Volume One*). The Panel understands that in the future, the Council, with the assistance of scientists knowledgeable in areas of research supported by the NEI, will provide more detailed estimates for the remaining years of the Plan based on actual budgetary experience and ongoing analyses of research progress. In this way the plan will be modified as necessary on a year-to-year basis.

At the end of each chapter in this report, subprogram tables show how the estimates shown in the following summary table have been derived from estimates for each research category included in each subprogram's Program Base and the Program Development Priorities.

SUMMARY RESOURCE TABLE

(Dollars in Thousands)

Subprograms	FY 1981 Grants* Cost		Panel Recommendation FY 83 Add. Grants Cost		Total Grants Cost**	
1. VASCULAR, INFLAMMATORY, AND NEOPLASTIC DISORDERS OF THE RETINA AND CHOROID						
a. Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities	51 $5,127	(13%)	9 $1,131	(8%)	60 $6,300	(12%)
b. Inflammatory Disorders	14 $1,558	(4%)	11 $1,067	(10%)	25 $2,625	(5%)
c. Tumors	18 $1,887	(5%)	11 $1,158	(10%)	29 $3,045	(6%)
2. DEGENERATIVE DISORDERS OF THE RETINA						
a. Developmental and Hereditary Disorders	42 $3,809	(11%)	17 $2,386	(15%)	59 $6,195	(12%)
b. Macular Degeneration	18 $1,418	(5%)	16 $2,152	(14%)	34 $3,570	(7%)
c. Retinal Detachment and Vitreous Disorders	18 $1,722	(5%)	9 $1,113	(8%)	27 $2,835	(5%)
d. Toxic and Environmental Disorders	2 $91	(1%)	2 $329	(2%)	4 $420	(1%)
3. FUNDAMENTAL PROCESSES AND RETINAL DISORDERS						
a. Retinal Pigment Epithelium	24 $1,931	(6%)	13 $1,954	(12%)	37 $3,885	(8%)
b. Photoreceptors, Visual Pigments, and Phototransduction	112 $9,514	(29%)	0 $2,246	(0%)	112 $11,760	(23%)
c. Retinal Organization, Neurotransmission, and Adaption	64 $4,729	(16%)	7 $2,726	(6%)	71 $7,455	(14%)

* Includes R01, R10, R23, P50, K04, and K07 mechanisms.

** Estimated average cost of grants in Retinal and Choroidal Diseases program for FY 1983 is $105,000.

SUMMARY RESOURCE TABLE

(Dollars in Thousands)

Subprograms/Areas	FY 1981 Grants* Cost		Panel Recommendation FY 83 Add. Grants Cost		Total Grants Cost**	
3. FUNDAMENTAL PROCESSES AND RETINAL DISORDERS (Continued)						
d. Glial Cells and the Retinal Microenvironment	6 $437	(1%)	3 $508	(3%)	9 $945	(2%)
e. Rescue and Regeneration of Neurons in the Optic Nerve and Retina	2 $136	(1%)	5 $599	(5%)	7 $735	(1%)
4. RELATED AREAS OF RESEARCH OPPORTUNITY AND NEED						
a. Noninvasive Techniques in the Study of Retinal Disorders	10 $1,176	(3%)	8 $714	(7%)	18 $1,890	(4%)
b. Tissue Acquisition and Distribution: Human Donor Eyes and Animal Models	[0] —		[6] —	***	[6] —	***
Total	**381** **$33,535**	**(100%)**	**111** **$18,083**	**(100%)**	**492** **$51,660**	**(100%)**

* Includes R01, R10, R23, P50, K04, and K07 mechanisms.

** Estimated average cost of grants in Retinal and Choroidal Diseases program for FY 1983 is $105,000.

*** Grants counted elsewhere within Retinal and Choroidal Diseases.

VASCULAR, INFLAMMATORY, AND NEOPLASTIC DISORDERS OF THE RETINA AND CHOROID

1

DIABETIC RETINOPATHY, SICKLE CELL RETINOPATHY, AND OTHER VASCULAR ABNORMALITIES

INTRODUCTION

THE RETINA HAS tremendous requirements for glucose, oxygen, and other nutrients that must be supplied by a well-functioning vascular system. Unfortunately, diseases of the retinal and choroidal vascular systems are common, and they are major causes of visual disability and blindness.

Diabetic retinopathy, a frequent complication of diabetes, is the most important disease of the retinal vasculature. Estimates by the National Society to Prevent Blindness,[1] based on unpublished data from the Model Reporting Area for Blindness Statistics, list diabetic retinopathy as the fifth leading cause of blindness, accounting for approximately 4,700 new cases in 1978 and an estimated 32,600 existing cases in that year. Diabetic retinopathy is the leading cause of new cases of blindness among persons aged 20 to 74 years, and among individuals aged 45 to 74 years, it is the second leading cause of blindness. Diabetes accounted for about 12.5 percent of new cases of blindness in 1978, and other vascular diseases of the retina an additional 2.2 percent. The other retinal vascular diseases that are important causes of visual disability are retrolental fibroplasia, sickle cell retinopathy, retinal vein occlusions, and hypertensive and atherosclerotic vascular disease.

New blood vessel formation arising from the choroidal capillaries may cause substantial damage in aging-related maculopathy and other macular disorders, which are discussed elsewhere (see Chapter 5, "Macular Degeneration"). Major occlusive disease of the choroidal vascular supply is infrequent, but causes extensive retinal damage when it occurs. In addition, the vascular supply to the optic nerve, derived from the choroidal system, may be interrupted in anterior ischemic optic neuropathy.

Clinical observations, including serial examinations with the ophthalmoscope, sequential retinal photography, and fluorescein angiography (see below), have indicated at least three major abnormalities that in various combinations characterize all of the retinal vascular diseases. They are *retinal vascular leakage*, *retinal vascular closure*, and *new blood vessel formation (neovascularization)*. Further elaboration of these abnormalities follows, prefaced by a brief description of the normal blood supply to the retina.

The retina is supplied by two major systems of blood vessels. Its inner layers of nerve and supporting (glial) cells are supplied by the *retinal circulation*, which in humans consists of branches of one main feeder trunk, the central retinal artery, and one main collecting trunk, the central retinal vein. Branches of these major vessels are arterioles, venules, and the smallest blood vessels of all, the capillaries, which have a caliber only sufficiently wide to allow passage of blood cells in single file (Figure 1).

The second circulatory system, the *choroidal circulation*, nourishes the outer layer of cells of the neural retina (the rods and cones) and the retinal pigment epithelium. This system consists of the three layers of choroidal vessels. The innermost vascular layer, the choroidal capillaries (or choriocapillaris), is adjacent to the pigment epithelium. These capillaries are of wider caliber, and are much more closely packed than are the capillaries of the

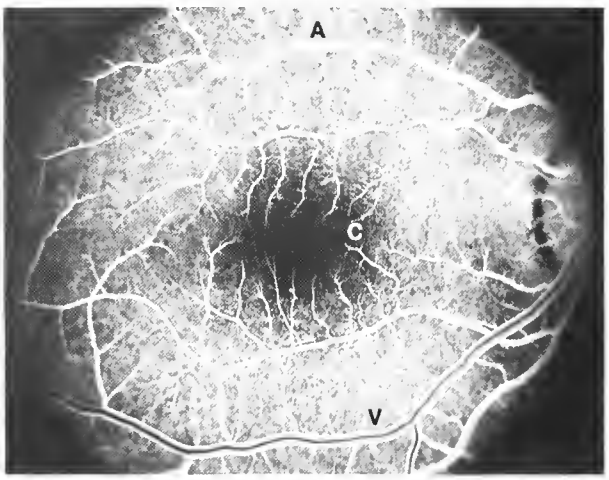


FIGURE 1. A frame from a fluorescein angiogram study of a normal subject. The arteries (A) have filled early in the study and fluoresce uniformly. A vein (V) began to fill later and has a striped appearance with two peripheral, fluorescent layers of blood surrounding a central, dark (nonfluorescent) layer. This "laminar flow" pattern is typical of retinal venous filling. Fine capillaries (C) surround the central, dark foveola which is devoid of blood vessels. The capillaries have a pattern much like a spiderweb. The mottled fluorescence beneath these retinal vessels comes from blood filling the choroidal capillaries. The mottled appearance is caused in part by melanin pigment in the retinal pigment epithelium, which partially blocks the choroidal capillary fluorescence, particularly in the center of the fovea.

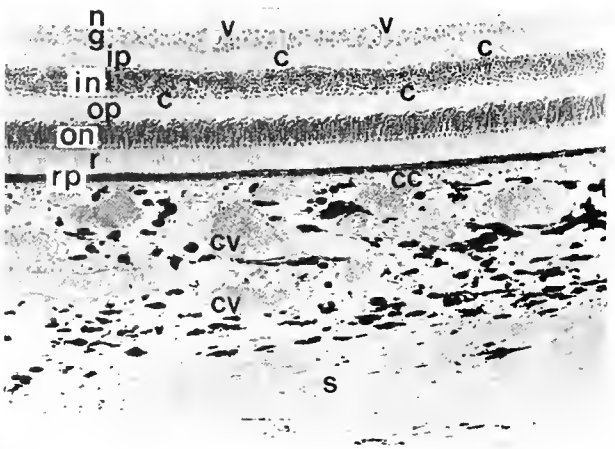


FIGURE 2. A cross section through a normal human retina as viewed through a microscope. The various cell layers of the retina and adjacent tissues are indicated: nerve fiber layer (n); ganglion cell layer (g); inner plexiform layer (ip); inner nuclear layer (in); outer plexiform layer (op); outer nuclear layer (on); retinal receptor layer (r) (rods and cones); retinal pigment epithelium (rp); sclera (S). Blood vessels are found in all retinal layers from the inner nuclear layer to the nerve fiber layer. Larger vessels (in this case, venules) are indicated by (V), while capillaries, barely visible in this photograph, are indicated by (c). The choroid is a richly vascular tissue. The fine capillary vessels of the choroid (cc) occupy a single layer just beneath the retinal pigment epithelium. Two layers of larger choroidal vessels (cv) lie beneath the capillaries. (Magnification: X250.) (Courtesy of Karni W. Frank, M.D., Department of Pathology, Wayne State University School of Medicine.)

retina, and their volume flow of blood is much greater than is that in the retinal circulation. Moreover, by electron microscopy, it can be shown that their lining cells (endothelial cells) have multiple holes (fenestrae) to allow ready passage of even rather large molecules. By contrast, the retinal vessels have a tight endothelial lining, which normally serves as a barrier to many substances in the bloodstream. External to the choriocapillaris are two additional layers of choroidal vessels, consisting of arteries and veins (Figure 2).

Normally, a tight barrier prevents the free exchange of blood components between the circulatory system and other retinal tissues. This blood-retinal barrier is formed by the endothelial cells lining the retinal blood vessels and by the cells of the retinal pigment epithelium, and appears to be essential for normal retinal function. Disruption of this barrier occurs in diabetic retinopathy, retinal vein occlusions, and other diseases. Gross leakage of fluid and molecules into the retina results in edema of the macula, with substantial loss of central vision.

An important goal is to determine the cellular mechanisms by which these leaks occur and to discover means to prevent them. Possible mechanisms include a breakdown of the specialized tight junctions between vascular endothelial cells and between cells of the retinal pigment epithelium (Figure 3) (see Chapter 8, "Retinal Pigment Epithelium"), a pathologic increase in bulk transfer of molecules by the process of pinocytosis (Figure 4), an alteration of specialized molecular pumps in cell membranes, or simple anatomical alterations in endothelial cells such that they become thin and fenestrated (Figure 5).

Closure of retinal vessels may be expected to cause major alterations in retinal function. Occlusion of the central retinal artery, or of one of its branches, usually by an atheromatous embolus released from the heart or carotid arteries, may cause sudden, severe visual loss. Occlusion of the central retinal vein, or of one of its branches, may also cause rapid diminution of vision, ranging from moderate to very severe. Later sequelae of retinal venous occlusion may include retinal neovascularization and hemorrhage or, particularly with occlusion of the central retinal vein, neovascularization of the anterior segment of the eye. This latter condition can bring about a decrease in aqueous humor drainage and intractable glaucoma.

Closure of retinal capillaries does not produce such dramatic symptoms, but the long-term effects may be almost as harmful to vision. The combination of retinal fluorescein angiography with special digest preparations of retinal blood vessels, obtained postmortem or after surgical enucleation of the eye, has shown that retinal capillaries become functionally occluded when they have lost all of their constituent cells (endothelial cells and pericytes) and become simply empty tubes of basement membrane (Figure 6). Alternatively, microthrombi composed of blood platelets and fibrin may occlude capillaries. Because the retina has the highest

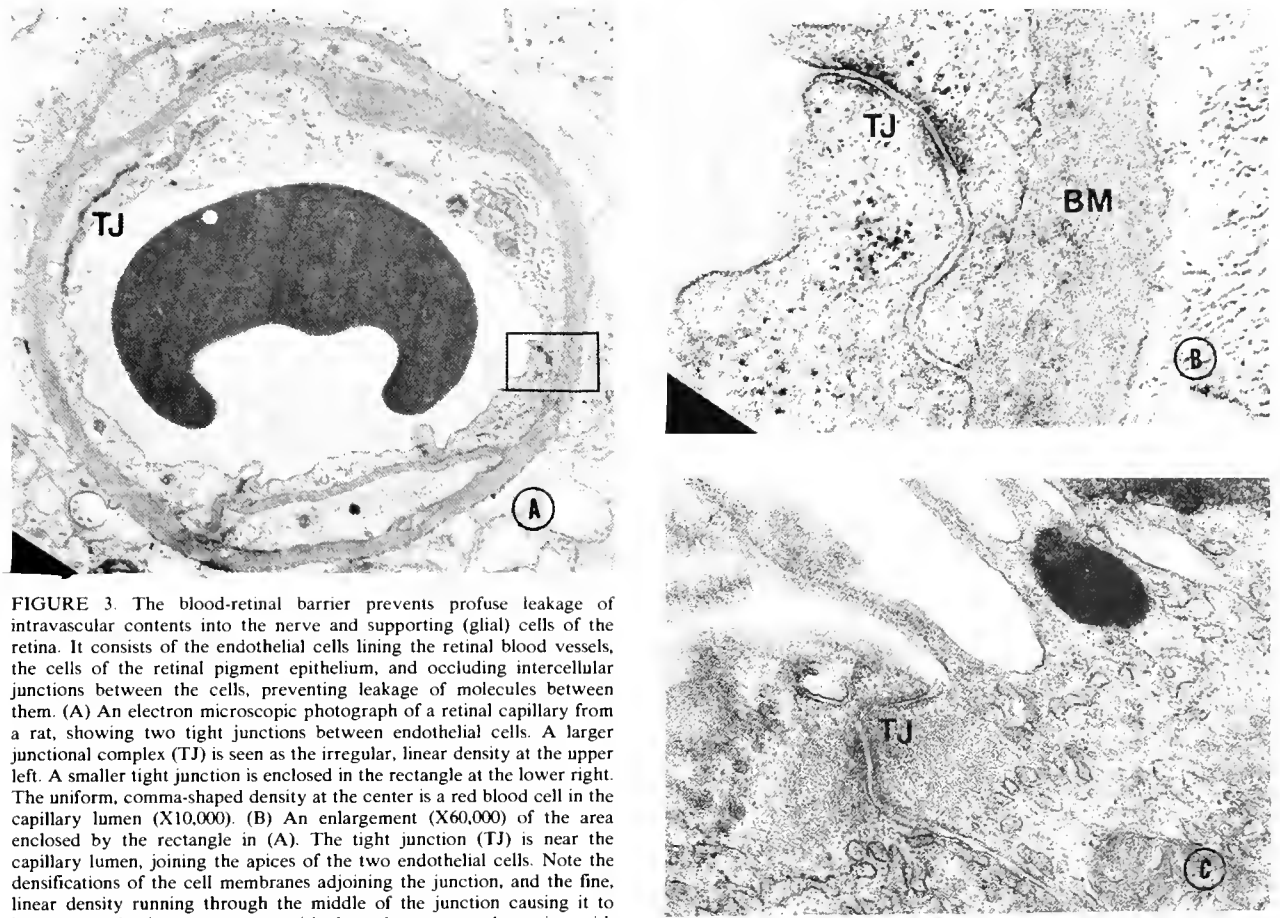


FIGURE 3. The blood-retinal barrier prevents profuse leakage of intravascular contents into the nerve and supporting (glial) cells of the retina. It consists of the endothelial cells lining the retinal blood vessels, the cells of the retinal pigment epithelium, and occluding intercellular junctions between the cells, preventing leakage of molecules between them. (A) An electron microscopic photograph of a retinal capillary from a rat, showing two tight junctions between endothelial cells. A larger junctional complex (TJ) is seen as the irregular, linear density at the upper left. A smaller tight junction is enclosed in the rectangle at the lower right. The uniform, comma-shaped density at the center is a red blood cell in the capillary lumen (X10,000). (B) An enlargement (X60,000) of the area enclosed by the rectangle in (A). The tight junction (TJ) is near the capillary lumen, joining the apices of the two endothelial cells. Note the densifications of the cell membranes adjoining the junction, and the fine, linear density running through the middle of the junction causing it to have a pentalaminar appearance with three dense zones alternating with two lighter ones. BM = capillary basement membrane. (C) A section near the apices of two retinal pigment epithelial cells from a rat, showing a junctional complex between them. TJ = tight junction (X50,000). (Figure 3C courtesy of Edward Essner, Ph.D., Kresge Eye Institute, Wayne State University School of Medicine.)

oxygen requirement of any tissue in the body, as well as high requirements for glucose and other metabolites, loss of the blood supply even to a small region of retina may be expected to have serious consequences. One important current hypothesis, amplified below, is that extensive areas of retinal capillary nonperfusion may in some way stimulate the growth of abnormal new blood vessels. Capillary nonperfusion may be demonstrated in diabetic retinopathy, sickle cell retinopathy, retinal vein occlusions, retrolental fibroplasia, and other diseases. Current research is attempting to discover the causes of retinal capillary cell death in diabetes and other diseases, to elucidate the relationship between altered platelet aggregation and diabetic retinopathy, and to determine if normalizing this increased aggregability can prevent the retinopathy from progressing. Attempts are also being made to produce models of capillary nonperfusion in the retinal circulation of laboratory animals.

Closure of choroidal vessels, a less commonly recognized condition, may also have devastating consequences. This is particularly true when the nutrient vessels to the optic nerve, derived from the posterior ciliary arteries (a part of the choroidal circulation), become impaired. Such choroidal vascular disease occurs in anterior ischemic optic neuropathy, a disorder of unknown cause, which frequently results in severe loss of vision in the elderly. Anterior ischemic optic neuropathy is often found in cranial (temporal) arteritis, in which an inflammatory process affects the small arteries of the scalp, brain, and optic nerve. If it is detected early, cranial arteritis may be treated with corticosteroids and vision salvaged, but there is no effective treatment for other forms of anterior ischemic optic neuropathy.

New blood vessel formation from the retinal and choroidal circulations may be a serious consequence of many eye diseases.[2] Retinal neovascularization occurs in diabetic retinopathy, sickle cell retinopathy, retinal vein occlusions, and retrolental fibroplasia, to name just the most important causes. Choroi-

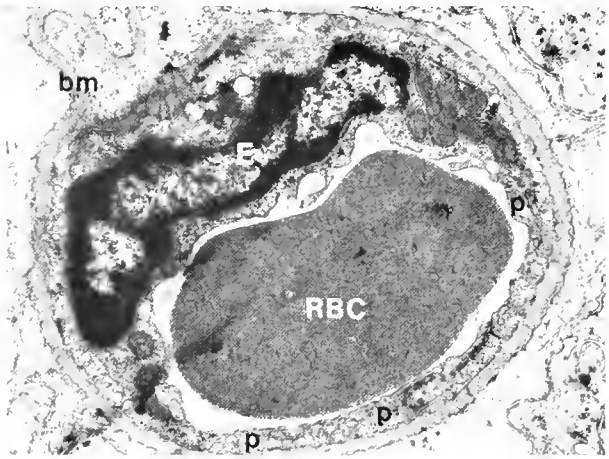


FIGURE 4. An electron micrograph of a retinal capillary from a rat showing multiple micropinocytotic vesicles (p), which appear as round, lucent vacuoles throughout the endothelial cell cytoplasm. These appear to be a mechanism for transporting molecules across the blood-retinal barrier, and in this particular cell, they are very numerous. E = endothelial cell nucleus. bm = capillary endothelial basement membrane. RBC = red blood cell within the capillary lumen (X15,000).

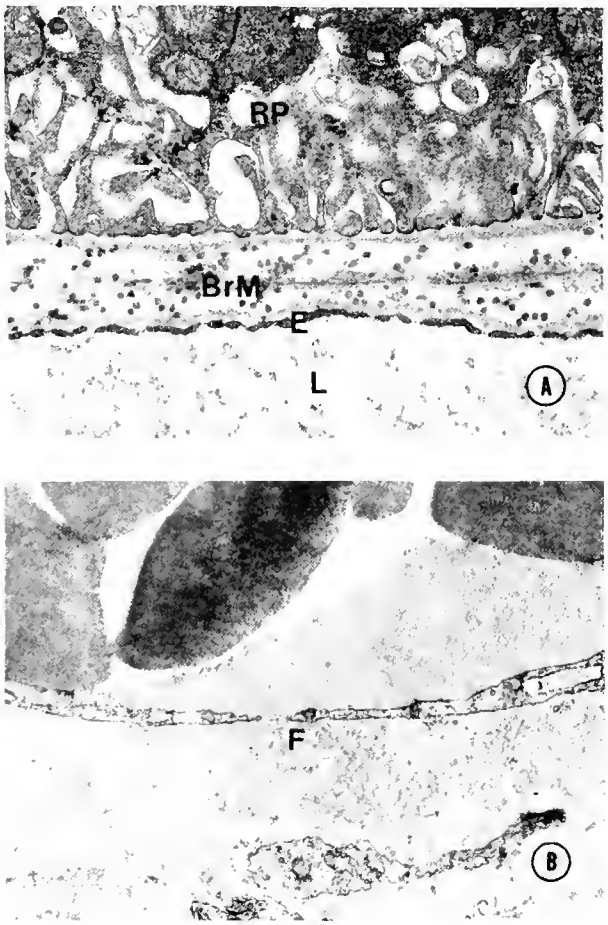


FIGURE 5. The endothelial lining of blood vessels in the retina and choroid is not always a thick barrier to the passage of molecules. In some locations it is normally porous, and at other times it becomes so as a result of disease. (A) The basal portion of a retinal pigment epithelial cell from a rat (RP). Underlying the cell is Bruch's membrane, (BrM), beneath which is the layer of choroidal capillaries. The lumen of one such large capillary is indicated by L. Note the thin, attenuated endothelial lining (E) with multiple pores, or fenestrae (X50,000). (Courtesy of Edward Essner, Ph.D., Kresge Eye Institute, Wayne State University School of Medicine.) (B) Under abnormal circumstances, the endothelial lining of retinal vessels becomes attenuated. This vein was present in a neovascular membrane in an eye that had chronic, severe granulomatous inflammation. Note the fenestration (F). Multiple red blood cells are seen in the vessel lumen in the upper half of the photograph (X50,000). (Courtesy of Karni W. Frank, M.D., Department of Pathology, Wayne State University School of Medicine.)

dal neovascularization occurs in aging-related maculopathy, the presumed ocular histoplasmosis syndrome, and in several other macular diseases. The blinding consequences of retinal neovascularization may be hemorrhage into the vitreous and the formation of fibrous scar tissue surrounding the new vessels. The fibrous tissue may contract, causing the retina to become detached. Hemorrhage from choroidal neovascularization causes marked loss of central vision, which often becomes permanent after formation of the typical circular (disciform) scar underneath the central retina. Current research is primarily aimed at uncovering the cellular and chemical mechanisms underlying neovascularization, with the hope that once these mechanisms are understood, better methods will be found to treat and prevent new vessel formation.

Research on retinal and choroidal vascular diseases benefits greatly from interactions with other fields of vision research and with biomedical research in general. Within vision research, there is an obvious close relationship to studies of the physiology and metabolism of the retina and retinal pigment epithelium. Investigations of major systemic diseases that cause vascular disorders in the eye have immediate relevance: diabetes, the sickle cell hemoglobinopathies, and atherosclerosis, to name the most common. In addition, research in tissue culture and growth factors dealing with a number of cellular systems, and supported by other NIH Institutes and other agencies, is directly related to this subprogram as are hematologic studies of platelet and other blood cell functions, and studies of the physiology and cell biology of blood vessels.

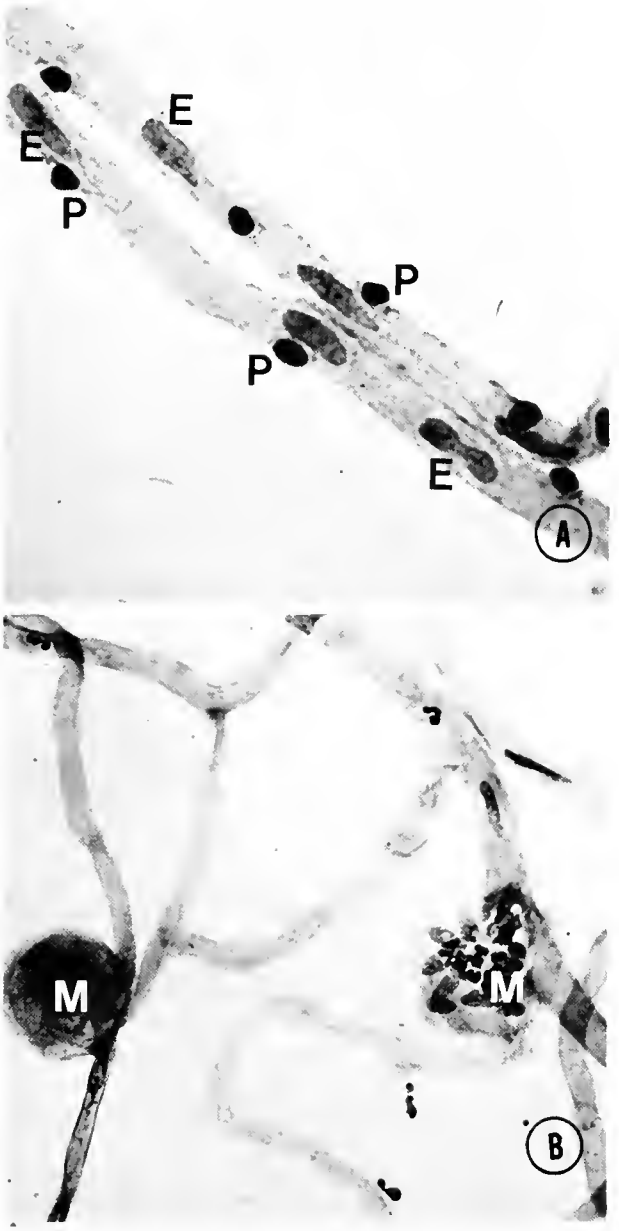


FIGURE 6. A trypsin digest preparation of capillaries from a normal human retina. (A) Endothelial cell nuclei (E) are seen as oval, lightly staining structures with their long axes running parallel to the course of the capillary. Intramural pericyte nuclei (P) are small, round, densely-staining structures that appear to bulge out of the capillary tube like "bumps on a log." In a normal human retinal capillary network, there are approximately equal numbers of pericytes and endothelial cells (X500). (B) A trypsin digest preparation from the retina of a diabetic patient. Many of the capillaries are empty tubes devoid of cell nuclei. A few endothelial cell nuclei remain. Microaneurysms (m) are bulbous enlargements of the capillaries. Some are totally lacking in cell nuclei while others contain multiple nuclei (X500). (Figures 6A and 6B courtesy of Toichiro Kuwabara, M.D., National Eye Institute.)

SUBPROGRAM OBJECTIVES

- To develop better methods of preventing, diagnosing, and treating diabetic retinopathy and other vascular diseases of the retina and choroid.
- To understand the anatomy, biochemistry, and physiology of the retinal and choroidal vasculature in normal and diseased states.
- To understand how blood flow is controlled in the retina.

OVERVIEW OF CURRENT RESEARCH SUPPORT

In fiscal year 1981, 51 grants were supported by the National Eye Institute, at a total cost of $5,127,000, in the area of diabetic retinopathy and other vascular and circulatory abnormalities of the retina and choroid, including six projects of the multicenter Branch Vein Occlusion clinical trial. Several grants dealing directly or indirectly with retinal and choroidal vascular diseases were funded by other NIH Institutes and other government agencies, for example, the Veterans Administration. In addition, the National Eye Institute currently supports contracts with 17 clinical centers for the Diabetic Retinopathy Vitrectomy Study, 23 clinical centers for the Early Treatment Diabetic Retinopathy Study, as well as a coordinating center and a photographic reading center for each study, and a central clinical laboratory for the Early Treatment Diabetic Retinopathy Study.

Additional support for research in retinal and choroidal vascular diseases is provided by several private agencies, including Fight for Sight, Inc., Research to Prevent Blindness, the National Society to Prevent Blindness, and in the area of diabetic retinopathy, the Juvenile Diabetes Foundation, the American Diabetes Association, and the Kroc Foundation.

RECENT ACCOMPLISHMENTS

Specific Diseases

Diabetic Retinopathy. Visual disability from the proliferative form of diabetic retinopathy (in which

new blood vessels grow from the retinal circulation) can be sharply reduced by extensive retinal photocoagulation with the xenon arc or the argon laser, as demonstrated by the nationwide Diabetic Retinopathy Study and discussed in the Council's 1977 Plan. The success of this major clinical trial has led to the initiation of two additional collaborative clinical trials in diabetic retinopathy. These are the Diabetic Retinopathy Vitrectomy Study (DRVS), now in its fifth year, and the Early Treatment Diabetic Retinopathy Study (ETDRS), which began recruiting patients in early 1980.

The DRVS is attempting to learn, first, if vitrectomy surgery (in which the contents of the vitreous cavity are removed and replaced with a balanced salt solution) is best done within a month after acute, severe vitreous hemorrhage in diabetic patients, or is preferably delayed at least six months. Second, the DRVS is attempting to determine if vitrectomy for diabetics threatened with detachment of the macula by fibrous traction improves the prognosis for vision.

The ETDRS is the most ambitious clinical trial yet attempted in ophthalmology. It involves 23 clinical centers, a coordinating center, a photographic reading center, and a central clinical laboratory, and it plans to recruit over 4,000 patients. The goal of the ETDRS is to determine whether argon laser treatment and/or aspirin can effectively prevent progression of diabetic retinopathy at the preproliferative stage, thus improving the prognosis over the earlier results of the Diabetic Retinopathy Study, and also whether laser treatment and/or aspirin are effective in preserving or improving vision in patients with diabetic macular edema.* In addition to these major goals, the ETDRS will attempt to evaluate a number of prognostic factors as determined by medical history, physical examination, and laboratory tests, and determine the effects of laser therapy on several measures of visual function in addition to visual acuity measurements.

Sickle Cell Retinopathy. Patients with sickle cell hemoglobin (including about 10 percent of American blacks) are at risk of developing retinopathy, which may result in capillary closure and eventual development of neovascularization, hemorrhage, traction detachment of the retina arising in the retinal periphery, and vascular occlusions in the central retina with resultant visual loss. Individuals with hemoglobin SC disease, in which about half of the hemoglobin is type S (sickle cell hemoglobin) and half is type C (another abnormal variant), are at particularly high risk. Currently, a clinical trial involving two institutions, the University of Illinois and the University of the West Indies in Kingston, Jamaica, is attempting to determine the efficacy of laser photocoagulation in arresting the progression of peripheral retinal neovascularization in sickle cell retinopathy. This study is supported by the Sickle Cell Disease program of the National Heart, Lung, and Blood Institute. In another development, investigators at the Comprehensive Sickle Cell Center at Wayne State University and at the University of Tennessee have described a deficiency of the trace metal zinc in some patients with sickle cell anemia (hemoglobin SS disease). Although these patients may have no signs of sickle cell retinal vascular disease, their retinal rod and cone cells have a diminished capacity to regain sensitivity following exposure to a bright "adapting" light. This diminished dark adaptation can be restored by supplemental zinc therapy, presumably because zinc is a cofactor for an enzyme needed to regenerate visual pigment molecules.[3]

Retrolental Fibroplasia (Blindness of Prematurity). Studies in the 1950s that showed the adverse effects of excessive oxygen on the retinal blood vessels of premature infants[4] provided the means to reduce substantially the incidence of this condition. However, the disease has not been eliminated, in part because no technique has been perfected to establish how much oxygen is actually reaching the infant eye on a minute-to-minute basis, and in part because, with increased survival of very small premature infants of very short gestation time, many of whom require delicate monitoring of oxygen delivery, the risk of developing retrolental fibroplasia has dramatically increased. Antioxidants such as vitamin E are currently being investigated in view of preliminary data suggesting a possible beneficial role.[5] Attempts to control the proliferative stage of retrolental fibroplasia will benefit from research on angiogenesis, and on the inhibition of the neovascular process (see below).

Retinal Vein Occlusions. A cooperative clinical trial supported by NEI involving six centers is investigating the value of argon laser photocoagulation in controlling the major complications of branch retinal vein occlusion, macular edema, and retinal neovascularization with hemorrhage. Treatment of the far more serious central retinal vein occlusion remains enigmatic. Panretinal photocoagulation appears to reduce the incidence of neovascular glaucoma,[6] a dreaded sequel both of central retinal vein occlusion and proliferative diabetic retinopathy, but this treatment does not improve the prognosis for vision. A relatively small clinical trial in Great Britain used fibrinolytic agents (which dissolve blood clots) and reported a modest improvement in visual prognosis in patients with

* Aspirin is thought to be beneficial in diabetic retinopathy for two reasons. First, it has been claimed that patients with diabetes and also other diseases (for example, arthritis) who require large aspirin doses have a lower than expected prevalence of diabetic retinopathy. Second, many diabetic patients have increased aggregability of blood platelets, which is thought to lead to small vessel occlusions and eventually to retinopathy. Aspirin is known to inhibit platelet aggregation.

central retinal vein occlusion.[7] There has been interest in beginning a similar trial, on a larger scale, in the United States.

Retinal Artery Occlusion. Occlusion of the central retinal artery is followed by immediate blindness. Occlusion of a branch retinal artery produces immediate visual loss, the severity of which is determined by the size of the occluded vessel and the proximity of the region of the retina it supplies to the macula. Retinal artery occlusions represent serious ocular emergencies, for unless effective treatment is initiated promptly, the resultant loss of vision is irreversible. A recent study has indicated that if the retinal circulation is restored within 97 to 100 minutes, vision can return.[8] Unfortunately, this tolerance time is often too little for patients who do not immediately recognize the seriousness of their disease, or who cannot rapidly obtain medical care. Even for those who do, treatment is not always effective.

Advances in Methodology and Instrumentation

The accomplishments described below, all of which occurred during the last few years, represent important milestones toward conquering retinal vascular disease (see Chapter 13, "Noninvasive Techniques in the Study of Retinal Disorders"). A major advance has been the development of vitrectomy, a new surgical technique that has restored at least partial vision to individuals who would otherwise have been blind. For the most part, however, accomplishments in this area involve the development of innovative methods that hold great promise for answering questions about the mechanisms of disease. Only time and much effort will show whether these methods will fulfill their promise.

Vitrectomy. In this procedure, specially designed instruments are inserted into the eye through tiny incisions just anterior to the boundary of the peripheral retina.[9-11] Using an operating microscope and fiberoptics light sources, the surgeon and his assistants can visualize the interior of the eye to remove hemorrhages, attempt to repair retinal detachments caused by traction from proliferative bands or giant retinal tears, remove foreign bodies, and strip fibrous membranes from the inner surface of the retina that, by puckering the retina, have decreased vision. For diabetic patients with blindness due to vitreous hemorrhage or traction detachment of the retina, useful vision may be restored in some 50 to 70 percent of selected cases.[12-14] However, as noted above, there are questions regarding the optimal time to use vitrectomy for diabetic vitreous hemorrhage and for traction that threatens macular vision but has not yet caused substantial visual loss. It is expected that the Diabetic Retinopathy Vitrectomy Study will provide answers to these questions. Other aspects of vitrectomy are discussed in Chapter 6, "Retinal Detachment and Vitreous Disorders."

Vitreous Fluorophotometry and Computerized Image Processing of Fluorescein Angiograms. Fluorescein angiography has been helpful in diagnosing and treating many retinal vascular diseases. It has been used to demonstrate the abnormal leakiness of retinal vessels and the presence of nonperfused capillary zones in certain conditions. It has been reported, for example, that in young diabetics with no other detectable retinal abnormalities, fluorescein leakage may be detected photographically during a fluorescein angiogram.[15] Vitreous fluorophotometry and computerized image processing of videotaped fluorescein angiograms are offshoots of the original technique. In vitreous fluorophotometry, a light source to stimulate fluorescence is mounted on a slit lamp, and the fluorescence emitted by the dye that has leaked into the vitreous cavity at various times following intravenous injection is measured using a sensitive photomultiplier tube.[16,17] The striking result obtained in early experiments with this technique is that much more dye leaks into the vitreous in individuals with early diabetes whose blood sugar is not well controlled, but who do not otherwise show signs of retinopathy, than it does in comparable normal individuals, or in diabetics maintained in good control on insulin.[18,19] The meaning of this result, however, is unclear, since neither the location nor the mechanism for the fluorescein leak has been demonstrated, nor is it known how this phenomenon may relate to diabetic retinopathy, which may develop later.

Fluorescein angiograms may be recorded on videotape as well as on film. The videotaped image gives a kinetic picture of blood flow through the retina which may be analyzed by computerized image processing techniques.[20] Although much additional research needs to be done, this method promises to be of great value in the quantitative assessment of blood flow in various regions of the retinal circulation. Measurements of blood vessel caliber, which may also be possible through this technique, will allow precise experimentation on autoregulation of the retinal circulation and its alteration in certain disease states.

Laser Doppler Measurement of Retinal Blood Flow. In this technique, light from a laser strikes red blood cells in retinal vessels. Some of the light is reflected back from the red cells, detected by a photomultiplier tube, and its frequencies determined by a spectrum analyzer. Because of the Doppler effect, the frequency of the light waves incident on the red cells will be shifted in proportion to the velocities of the red cells. Hence, by measuring the

frequency shift, the velocity of the red cells can be calculated. [21–23]

Further improvements in this technique, and its application in various stages of retinal vascular disease and in individuals, such as diabetics, who are at high risk for the development of retinal vascular disease, should provide substantial new information about blood flow in the retina and its role in the development of diseases. For example, differences in serum viscosity have been shown to exist in diabetics with and without small vessel disease in the eye, nervous system, and kidney. [24] Other data suggest that there are increases in red cell aggregation [25] in diabetic microangiopathy and that diabetic red blood cells have a decreased deformability, that is, their membranes are less elastic. [26] These and other factors, such as increased platelet aggregation in diabetes, [27] may alter blood flow in the retinal vessels of individuals with diabetes and change the shear stress on the endothelial cells lining these vessels, resulting in damage to these cells. Although laser Doppler measurements of retinal blood flow and other studies noted in this section are still in a relatively early stage, further developments in these areas promise to be exciting.

Retinal Oximetry. Another technique still in a developmental stage is retinal oximetry, which attempts to measure oxygen saturation of blood in the vessels in various parts of the retinal circulation. The method uses a photoelectric system to measure the reflectance of a particular vessel at three different wavelengths, and to compare these measurements for the known values of oxygenated and deoxygenated hemoglobin. [28] Use of this method should add further knowledge of the retinal circulation and retinal metabolism.

Tissue Culture Studies of Retinal Vascular Cells: The Search for an Angiogenesis Factor. Some years ago, it was discovered that in the retinal capillaries of individuals with early diabetic retinopathy, the intramural pericytes degenerated more rapidly than did the endothelial cells. [29–31] The intramural pericytes are cells that reside in the basement membrane "skeleton" of the capillary outside the endothelial cell lining. Their function is not clear, but their selective loss early in the course of diabetic retinopathy may figure importantly in the later pathological alterations that occur. Within the past few years, it has become possible to grow in tissue culture both pericytes [32] and endothelial cells [33,34] from retinal capillaries (Figure 7). This development makes it possible to study the biochemical and physiological properties of these cells, and in particular compare them with similar cells from other vascular systems that are not so severely affected by diabetes. The results of initial biochemical investigations of cultures of retinal capillary pericytes have already been published. [35,36]

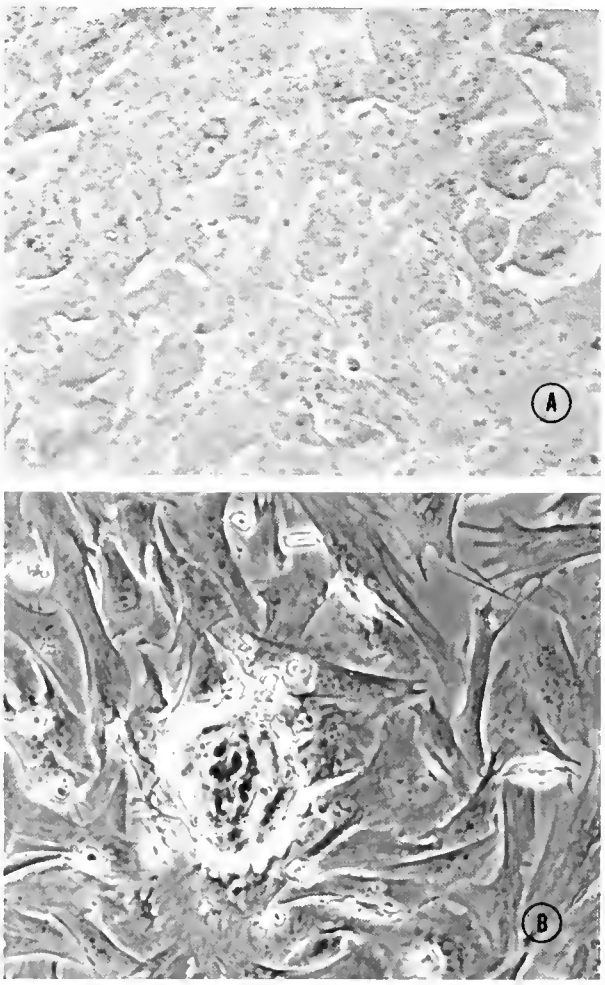


FIGURE 7. (A) Phase contrast photomicrograph of endothelial cells from fetal pig retinal capillaries growing in tissue culture. Note that they grow as a single layer of cells in a hexagonal, mosaic-like arrangement (X200). (B) Phase contrast photomicrograph of pericytes from calf retinal capillaries in tissue culture. The cells grow as irregular polygons, sometimes partially overlapping one another, and often with spaces in between. The round structure just to the left of center is the remainder of the basement membrane of the original capillary from which the cells sprouted (X200).

Retinal neovascularization, as noted previously, is the most damaging manifestation of diabetic retinopathy and other retinal vascular diseases. Many years ago, it was suggested that the retina in certain pathological conditions produces an angiogenesis factor that stimulates abnormal vessel growth. [37,38] More recently, Folkman and his colleagues proposed that malignant tumors also produce angiogenesis factors that cause the luxuriant neovascular growth needed to support the vigorous metabolism of the tumor. [39,40] A point of common interest, therefore, in cancer research and in the study of

retinal vascular diseases is the isolation and characterization of these factors, if indeed they exist.

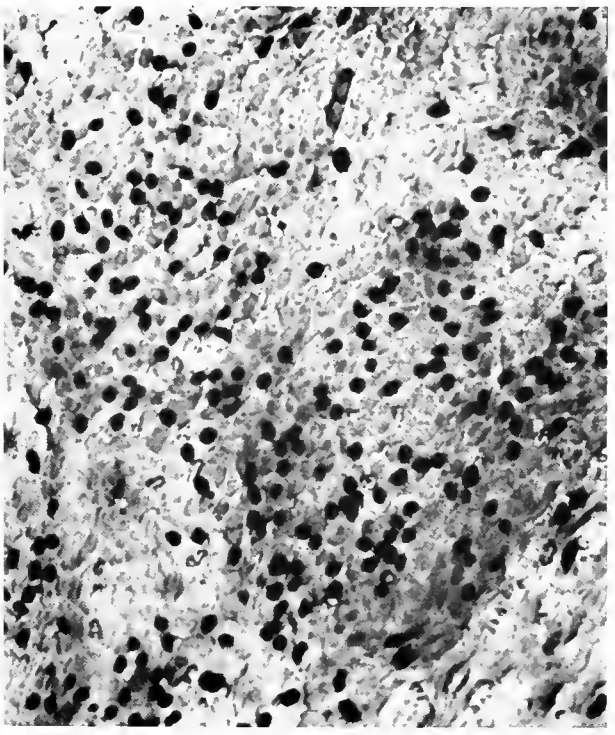


FIGURE 8. A monolayer of retinal capillary endothelial cells that had been growing in culture to which thymidine, labeled with tritium (a radioactive isotope of hydrogen), had been added. Thymidine is a molecule that is incorporated into DNA in the cell nucleus. Cells that are dividing will incorporate the labeled thymidine. If the culture is then fixed and coated with a liquid photographic emulsion, then allowed to incubate for a period in the dark, the dividing cells will emit radioactivity that develops the emulsion. The dark nuclei in the photograph are those that have incorporated the labeled thymidine and hence, are dividing. By counting the proportion of labeled to unlabeled nuclei, one can estimate how actively the culture is proliferating. This method then can be used, for example, to compare substances that might accelerate cell proliferation, such as presumed angiogenic factors (X100).

Until recently, a major difficulty in identifying a presumed angiogenesis factor was the absence of a physiologically valid, sensitive, and reproducible assay system. The development of methods for the culture of vascular endothelial cells appears to represent a suitable assay (Figure 8). A substance extractable from normal retinas of several species has been reported to stimulate proliferation in cultures of aortic endothelial cells.[41] Attempts to characterize this substance biochemically are under way. Capillary endothelial cells are much harder to maintain in culture than those from larger vessels, such as the aorta, but with the development of techniques for subculturing capillary endothelial cells,[42] it has been possible to use them for angiogenesis factor assays. By using extracts presumed to contain tumor angiogenesis factor activity, investigators have found that only capillary endothelial cells, and not those from the aorta or other large vessels, exhibit a proliferative response[42] or increased migratory activity,[43] which is presumed to precede cell division in newly growing vessels. Because of these recent developments, further progress in understanding the biochemistry and function of retinal vascular cells and the chemical stimuli to retinal neovascularization, should be forthcoming in the next few years.

Epidemiologic Studies. A recent epidemiologic survey of the Pima Indians in Arizona identified primary, systemic hypertension as a risk factor for developing diabetic retinopathy.[44] Other studies have suggested hormonal influences on diabetic retinopathy. For example, an investigation of the relationship of age and sex to diabetic blindness found a much lower prevalence in premenopausal women than in men of the same age. Prevalence rates became equal for older men and women, suggesting that female hormones may exert a protective effect.[45] A study of retinopathy among children, adolescents, and young adults with diabetes showed little retinopathy before the age of 15, and a sharp increase thereafter.[46] The increase was independent of the duration of diabetes. Similar results had been suggested earlier.[47] This indicates that hormonal influences related to puberty may also have an important role in the development of diabetic retinopathy.

Still another study[48] showed that the severity of retinopathy was inversely related to survival. Diabetic individuals with little or no retinopathy had a survival rate, over the seven-year duration of the study, similar to that of the general population. However, as the severity of retinopathy increased, survival decreased. At the present time, at least two major epidemiologic studies of diabetic retinopathy are in progress. In one, approximately 2,400 diabetic patients (of a total diabetic population of more than 14,000 individuals in southern Wisconsin) are being surveyed to determine the prevalence and severity of retinopathy, and to identify possible causal or aggravating factors. Another study, at the Joslin Diabetes Center in Boston, uses case-control methodology. A large group of diabetic patients with known, severe retinopathy is being surveyed for a variety of factors that may affect the development or progression of the retinopathy. Another group of diabetics, matched as nearly as possible for age, sex, and duration of diabetes, is also being surveyed for the same factors. Statistical comparisons will determine if major differences in these factors exist between the two groups; these differences could be related to the development of retinopathy. If strongly suggestive factors can be identified by such

studies, their relationship to diabetic retinopathy can be further tested by other methods.

RESEARCH NEEDS AND OPPORTUNITIES

Tissue Culture of Retinal Capillary Cells

Further investigation of the properties of isolated retinal capillary pericytes and endothelial cells may lead to an understanding of how these cells become abnormal in diabetic retinopathy and other retinal vascular diseases. Studies of the blood-retinal barrier [49] using cultured cells may involve investigations of how intercellular tight junctions form and are disrupted, [34] and investigations of pinocytosis. [50,51] Biochemical studies may involve further investigation of the sorbitol pathway, [35,52] which is responsible for the development of cataracts in diabetic and galactosemic animals of certain species (see *Volume Two, Part Three, Report of the Cataract Panel*) and may play an important role in diabetic retinopathy. Studies of collagen and basement membrane biosynthesis should also be pursued. [36,53]

An important problem in diabetic retinopathy is the fact that while the retinal vessels are affected, the structurally similar vessels of the cerebral cortex, an organ that is closely related developmentally to the retina, appear to be spared. [54] Hence, comparative studies of the capillaries of the retina and cerebral cortex should be emphasized.

Properties of cultured retinal vascular cells may have great value in retinal vascular diseases other than diabetes. For example, it has been found that sickled red blood cells from patients with sickle cell anemia adhere more readily to cultured aortic and umbilical vein endothelial cells than do normal red blood cells. [55,56] Similar experiments using cultured retinal vascular cells might be used to develop agents that would prevent such cellular adherence and thereby protect against sickle cell retinopathy.

Angiogenesis Factors

With the development of an assay system using cultured capillary endothelial cells, [33,34,42,43] a major difficulty in the study of angiogenesis factors may have been overcome. Because of the clinical importance of retinal neovascularization, this area of research should have high priority. A problem in these studies is that such factors may be present in only minute concentrations in the ocular fluids of diabetic patients and others with retinal vascular disease; hence, their identification and characterization may be difficult. However, similar doubts were expressed at one time about the possibility of isolating and characterizing trophic hormones from the brain, but persistence and ingenuity over a period of years resulted in the successful identification of several of these substances and tremendous benefit to medical science. [57,58]

Blood Flow and the Blood-Retinal Barrier

Further development of such methods as laser Doppler velocimetry and computer analysis of videotaped fluorescein angiograms should be emphasized as a potential means of developing precise, quantitative methods for the measurement of retinal blood flow. Methods of studying retinal blood flow in the past have led only to approximate results which have not adequately answered questions about regional differences in different parts of the retina, or differences in early diabetics as opposed to normals, which might relate to the eventual development of retinopathy. Techniques for the measurement of blood oxygenation (oximetry) in different segments of the retinal circulation should be refined to provide insights into a possible causal mechanism of retinal vascular disease.

The initial results of vitreous fluorophotometry studies, showing a breakdown of the blood-retinal barrier in early diabetes before retinopathy becomes clinically evident, are of great interest. This technique clearly has great importance, but it must first be standardized so that investigators in many centers will have a common basis for comparing their results. Using vitreous fluorophotometry and other methods in laboratory animals, further studies should be made of the site of the breakdown in the blood-retinal barrier. The initial assumption that it is located in the retinal vascular endothelial cells may not be correct; recent evidence suggests that the site of the breakdown instead may be at the level of the retinal pigment epithelium. [59] If so, this would be the first demonstration in diabetes of an abnormality of the retinal pigment epithelium, a tissue that has been thought to remain relatively unaffected by this disease.

Animal Models of Retinal Vascular Disease

Many animals develop diabetes spontaneously; in others, the disease can be induced by drugs, viral infections, or surgery. Hence, investigators are interested in determining whether animals with diabetes will develop retinopathy that can be studied as a model for the human disease. There is now good evidence that dogs with spontaneous or chemically induced diabetes of at least five years' duration develop retinal vascular lesions like those of human nonproliferative diabetic retinopathy. [60] In one study, a group of diabetic dogs was maintained with good blood sugar control while a comparable

group was intentionally maintained with poor control.[61] Animals with poor control regularly developed retinopathy after five years, while the lesions in the good control group were minimal.

Studies such as these have obvious therapeutic implications for humans. In addition, the availability of animals with diabetes, particularly those that develop diabetic retinopathy, permits a range of studies that cannot be conducted in humans aimed at learning more about causal mechanisms and potential therapies for this devastating eye disease. Because the dog model is the best one now available for diabetic retinopathy, it should be further studied. However, dogs with diabetes are expensive and difficult to maintain for the five years necessary for retinopathy to develop. Therefore, it is essential to produce animal models that are easier to develop and maintain. Furthermore, there are few animal models of proliferative retinopathy. The best is the retrolental fibroplasia model, obtained by placing newborn animals in a high oxygen atmosphere. These animals develop retinal neovascularization like human premature infants with retrolental fibroplasia.[62] Further investigation of this model, and development of new ones, will be useful for devising improved treatments for human proliferative retinopathies.

Retinal Vascular Occlusions

Further research is needed to find more effective treatment and to identify individuals who may be at greatest risk for developing retinal vascular occlusions. Although the problems are enormous, attempts should be made to prolong retinal survival time and restore function following vascular occlusions, and to revascularize or support artifically areas of retina whose blood supply has been impaired.

Therapeutic Trials

The success of the Diabetic Retinopathy Study and the subsequent enthusiastic support of the Diabetic Retinopathy Vitrectomy Study and the Early Treatment Diabetic Retinopathy Study by the ophthalmic community have demonstrated the value of large-scale clinical trials in testing new treatments for retinal vascular disease. Two other smaller clinical trials, the NEI-supported Branch Vein Occlusion Study and the Sickle Cell Retinopathy Photocoagulation Study, which is supported primarily by the National Heart, Lung, and Blood Institute, are now also under way. Additional therapeutic trials should be developed as promising new treatments are proposed for retinal vascular diseases. A study of potential major importance would be an effort to determine whether tight blood glucose control in diabetes is effective in retarding the development and progression of diabetic retinopathy and other complications of diabetes. The complexities of such a study would be formidable, but with the development of the portable insulin infusion pump,[63,64] an approach to nearly physiological control of blood glucose in insulin-requiring diabetics may be possible. An important precondition for a therapeutic trial is the determination of the approximate number of patients that will be required in the tight and standard control groups to produce a high likelihood of obtaining statistically significant results at the end of the study period, presumably five years. Investigations by several ophthalmic research groups have produced data that are in close agreement concerning the prevalence of retinopathy in juvenile-onset, insulin-dependent diabetics treated by standard methods for specific periods of time.[46,65] These data will be of substantial value in planning the proposed clinical trial.

Epidemiologic Studies

Investigations of the incidence and prevalence of retinal vascular diseases are of great importance, since these can lead to identification of causal factors. In addition to the studies in progress, others should be designed to investigate diabetic retinopathy and the various forms of retinal vascular occlusive disease.

RECOMMENDATIONS

Based on the foregoing assessment of recent accomplishments, current activities, and research needs and opportunities in "Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities," the Panel has made the following recommendations concerning research in this subprogram over the next five years. These have been grouped under two headings: Program Base and Program Development Priorities.

The Program Base includes areas of ongoing research where the current level of activity is considered adequate, or areas of ongoing research in which there may be great need for additional activity, but where, in the Panel's judgment, little or no opportunity (new methods or insights) exists at present to justify a significant expansion of effort. Nonetheless, additional applications for research grants in these areas may be funded if they are innovative and of very high quality as determined by the NIH peer review system.

Program Development Priorities include areas of ongoing research in which new knowledge and

techniques offer particular opportunities for scientific progress, or promising new areas of research in which there is little or no support at present but where there is both great need and high potential for success. Such areas are judged to warrant significantly increased support over the next five years, provided that high quality applications for research grants in these areas are forthcoming.

Program Base

- Characterize factors responsible for the growth of new retinal blood vessels.
- Study the location of and mechanisms responsible for the breakdown of the blood-retinal barrier in early diabetes, hypertension, and other retinal vascular diseases.
- Continue to support clinical trials of treatments for retinal vascular diseases. Develop new trials as promising new treatments become available. Collaborate with other agencies to develop clinical trials for testing the efficacy of rigorous blood sugar control in the prevention of diabetic retinopathy and other complications of diabetes.
- Encourage epidemiologic studies on various types of retinal vascular disease with a particular view to isolating causative factors.
- Further improve and evaluate methods for diagnosing and treating retinal vascular disorders. Such examples are laser Doppler velocimetry, vitreous fluorophotometry, rheology, and computerized analysis of videotaped fluorescein angiography (see Chapter 13).

Program Development Priorities

- Expand studies of the metabolism of retinal vascular cells and other endothelial cells using both freshly isolated specimens and cells grown in tissue culture.
- Develop new animal models of vasoproliferative retinopathies. Employ the existing dog model of diabetic retinopathy and new models to obtain additional information about the mechanisms and treatment of retinal vascular diseases.
- Investigate approaches that prolong retinal survival time after major vascular occlusion and permit restoration of retinal function after such an occlusion. Investigate methods for revascularizing areas of ischemic retina.
- Improve noninvasive methods for determining oxygen saturation in retinal blood vessels.
- Encourage basic research, which will lead to the pharmacological management of retinal vascular disorders.

RESOURCE REQUIREMENTS

After reviewing current research grant support in each of these categories and assessing the need and potential for future development, the Panel has estimated the number of projects it believes are needed to carry out its recommendations in FY 1983. These estimates are shown in the table on the following page. For a discussion of the general basis and significance of these projections, see the "Summary" at the beginning of this report.

RESOURCE TABLE

DIABETIC RETINOPATHY, SICKLE CELL RETINOPATHY, AND OTHER VASCULAR ABNORMALITIES

	No. of Grants FY 1981	Panel Recommendation FY 83	
		Add. Grants	Total Grants
Program Base			
A. Characterize factors responsible for growth of new retinal blood vessels.	3	0	3
B. Study location of and mechanisms responsible for breakdown of blood-retinal barrier in retinal vascular diseases.	13	−1	12
C. Continue to support clinical trials of treatments for retinal vascular diseases and test the efficacy of rigorous blood sugar control in the prevention of diabetic retinopathy.	10*†	0	10
D. Encourage epidemiologic studies on various types of retinal vascular disease.	4	0	4
E. Improve methods for diagnosing and treating retinal vascular disorders.	7	1	8
Program Development Priorities			
A. Expand studies of the metabolism of retinal vascular cells using isolated specimens and cells grown in tissue culture.	7	2	9
B. Develop new animal models of vasoproliferative retinopathies.	6	1	7
C. Investigate approaches that will prolong retinal survival time after major vascular occlusion and permit restoration of retinal function. Investigate methods for revascularizing areas of ischemic retina.	0	2	2
D. Improve noninvasive methods for determining oxygen saturation in retinal blood vessels.	1	2	3
E. Encourage basic research, which will lead to the pharmacological management of retinal vascular disorders.	0	2	2
Subtotal Grants	**51**	**9**	**60**
(% of Program)	**(13)**	**(8)**	**(12)**
Total Estimated Cost	**$5,127,000**	**$1,131,000**	**$6,300,000**

* Includes four single-center clinical trials: Photocoagulation Studies on Retinal Vein Occlusion; Prematurity, Vitamin E, and Retrolental Fibroplasia; Tocopherol Protection in Oxygen Induced Retinopathy; and Retrolental Fibroplasia: Clinical and Research Aspects; and six projects of the multicenter Branch Vein Occlusion Study.

† Does not include 2 contracts for the Diabetic Retinopathy Study; 11 contracts for the Diabetic Retinopathy Vitrectomy Study; 12 contracts for the Early Treatment Diabetic Retinopathy Study.

REFERENCES

1. *Vision Problems in the U.S.* New York, National Society to Prevent Blindness, 1980.
2. Henkind P: Ocular neovascularization. *Am J Ophthalmol* 85:287–301, 1978.
3. Warth JA, Prasad AS, Zwas F, et al: Abnormal dark adaptation and sickle cell anemia. *J Lab Clin Med* 8:189–194, 1981.
4. Kinsey VE: Retrolental fibroplasia. *Arch Ophthalmol* 56:481–543, 1956.
5. Phelps DL, Rosenbaum AL: Vitamin E in kitten oxygen-induced retinopathy: II. Blockage of vitreal neovascularization. *Arch Ophthalmol* 97:1522–1526, 1979.
6. May DR, Klein ML, Peyman GA: A prospective study of xenon arc photocoagulation for central retinal vein occlusion. *Br J Ophthalmol* 60:816–818, 1976.
7. Kohner EM, Pettit JE, Hamilton AM, et al: Streptokinase in central retinal vein occlusion: A controlled clinical trial. *Br Med J* 1:550–553, 1976.
8. Hayreh SS, Kolder HE, Weingeist TA: Central retinal artery occlusion and retinal tolerance time. *Ophthalmology* 87:75–78, 1980.
9. Machemer R, Parel JM, Buettner H: A new concept for vitreous surgery. *Am J Ophthalmol* 73:1–7, 1972.
10. Machemer R: A new concept for vitreous surgery: II. Surgical technique and complications. *Am J Ophthalmol* 74:1022–1033, 1972.
11. Machemer R, Norton EWD: A new concept for vitreous surgery: III. Indications and results. *Am J Ophthalmol* 74:1034–1056, 1972.
12. Michels RC: Vitrectomy for complications of diabetic retinopathy. *Arch Ophthalmol* 96:237–246, 1978.
13. Mandelcorn MS, Blankenship G, Machemer R: Pars plana vitrectomy for the management of severe diabetic retinopathy. *Am J Ophthalmol* 81:561–570, 1976.
14. Aaberg TM: Clinical results in vitrectomy for diabetic traction retinal detachment. *Am J Ophthalmol* 88:246–253, 1979.
15. Dorchy H, Toussaint D, Vanderschueren-Lodeweyck Y, et al: Leakage of fluorescein: First sign of juvenile diabetic retinopathy. *Acta Paediatr Scand (Suppl)* 277:47–53, 1979.
16. Cunha-Vaz JC, Abreu JRF, Campos AJ, et al: Early breakdown of the blood-retinal barrier in diabetes. *Br J Ophthalmol* 59:649–656, 1975.
17. Krupin T, Waltman SR, Oestrich C, et al: Vitreous fluorophotometry in juvenile-onset diabetes mellitus. *Arch Ophthalmol* 96:812–814, 1978.
18. Waltman SR, Krupin T, Hanish S, et al: Alteration of the blood-retinal barrier in experimental diabetes mellitus. *Arch Ophthalmol* 96:878–879, 1978.
19. Krupin T, Waltman SR, Scharp DW, et al: Ocular fluorophotometry in experimental diabetes mellitus in the rat: Effect of pancreatic islet isografts. *Invest Ophthalmol Vis Sci* 18:1185–1190, 1979.
20. McCormick BH, Read JS, Borouec RT, et al: Image processing in television ophthalmoscopy, in Preston K, Onoe M (eds): *Digital Processing of Biomedical Images.* New York, Plenum Press, 1976, pp 399–415.
21. Tanaka T, Riva C, Ben-Sira I: Blood velocity measurements in human retinal vessels. *Science* 186:830–831, 1974.
22. Feke GT, Riva CE: Laser Doppler measurements of blood velocity in human retinal vessels. *J Opt Soc Am* 68:526–531, 1978.
23. Riva CE, Feke GT, Eberli B, et al: Bidirectional LDV system for absolute measurement of blood speed in retinal vessels. *Appl Optics* 18:2301–2306, 1979.
24. McMillan DE: Plasma protein changes, blood viscosity and diabetic microangiopathy. *Diabetes* 25:858–864, 1976.
25. Little HL, Sacks AH: Role of abnormal blood rheology in the pathogenesis of diabetic retinopathy. *Trans Am Acad Ophthalmol Otolaryngol* 83:OP522–534, 1977.
26. McMillan DE, Utterback NC, La Puma J: Reduced erythrocyte deformability in diabetes. *Diabetes* 27:895–901, 1978.
27. Sagel J, Colwell JA, Crook L, et al: Increased platelet aggregation in early diabetes mellitus. *Ann Intern Med* 82:733–738, 1975.
28. Delori FC, Parker JS, Gragoudas ES: Oximetry of retinal vessels. *Invest Ophthalmol Vis Sci* 19(suppl):138, 1980.
29. Cogan DG, Toussaint D, Kuwabara T: Retinal vascular patterns: IV. Diabetic retinopathy. *Arch Ophthalmol* 66:366–378, 1961.
30. Speiser P, Gittelsohn AM, Patz A: Studies on diabetic retinopathy: III. Influence of diabetes on intramural pericytes. *Arch Ophthalmol* 80:332–337, 1968.
31. Yanoff M: Diabetic retinopathy. *New Engl J Med* 274:1344–1349, 1966.
32. Buzney SM, Frank RN, Robison WC Jr: Retinal capillaries: Proliferation of mural cells in vitro. *Science* 190:985–987, 1975.
33. Buzney SM, Massicotte SJ: Retinal vessels: Proliferation of endothelium in vitro. *Invest Ophthalmol Vis Sci* 18:1191–1195, 1979.
34. Frank RN, Kinsey VE, Frank KW, et al: In vitro proliferation of endothelial cells from kitten retinal capillaries. *Invest Ophthalmol Vis Sci* 18:1195–1200, 1979.
35. Buzney SM, Frank RN, Varma SD, et al: Aldose reductase in retinal mural cells. *Invest Ophthalmol Vis Sci* 16:392–396, 1977.
36. Cohen MP, Frank RN, Khalifa AA: Collagen production by cultured retinal capillary pericytes. *Invest Ophthalmol Vis Sci* 19:90–94, 1980.
37. Michaelson IC: The mode of development of the retinal vessels and some observations of its significance in certain retinal diseases. *Trans Ophthalmol Soc UK* 68:137–180, 1948.
38. Wise CN: Ocular neovascularization. *Trans Am Ophthalmol Soc* 54:729–826, 1956.
39. Folkman J, Merler E, Abernathy C, et al: Isolation of a tumor factor responsible for angiogenesis. *J Exp Med* 133:275–288, 1971.
40. Folkman J: The vascularization of tumors. *Scientific American* 234:58–73, 1976.

41. Glaser BM, D'Amore PA, Michels RC, et al: Demonstration of vasoproliferative activity from mammalian retina. *J Cell Biol* 84:298–304, 1980.
42. Folkman J, Haudenschild CC, Zetter BR: Long-term culture of capillary endothelial cells. *Proc Natl Acad Sci USA* 76:5217–5221, 1979.
43. Zetter BR: Migration of capillary endothelial cells is stimulated by tumour-derived factors. *Nature* 285:41–43, 1980.
44. Knowler WC, Bennett PH, Ballintine EJ: Increased incidence of diabetic retinopathy with elevated blood pressure. *New Engl J Med* 302:645–650, 1980.
45. Yuen KK, Kahn HA: The association of female hormones with blindness from diabetic retinopathy. *Am J Ophthalmol* 81:820–822, 1976.
46. Frank RN, Hoffman WH, Podgor MJ, et al: Retinopathy in juvenile-onset diabetes of short duration. *Ophthalmology* 87:1–9, 1980.
47. Knowles HC Jr: The course of juvenile diabetes treated with unmeasured diet. *Diabetes* 14:239–273, 1965.
48. Davis MD, Hiller R, Magli YL, et al: Prognosis for life in patients with diabetes: Relation to severity of retinopathy. *Trans Am Ophthalmol Soc* 77:144–170, 1979.
49. Cunha-Vaz JC: The blood-ocular barriers. *Surv Ophthalmol* 23:279–296, 1979.
50. Davies PF, Ross R: Mediation of pinocytosis in cultured arterial smooth muscle and endothelial cells by platelet-derived growth factor. *J Cell Biol* 79:663–671, 1978.
51. Au YK, Bellhorn MB: Quantitative evaluation of pinocytotic vesicles in retinal capillaries. *Invest Ophthalmol Vis Sci* 16(suppl):147, 1977.
52. Gabbay K: The sorbitol pathway and the complications of diabetes. *New Engl J Med* 288:831–836, 1973.
53. Howard BV, Macarak EJ, Gunson D, et al: Characterization of the collagen synthesized by endothelial cells in culture. *Proc Natl Acad Sci USA* 73:2361–2364, 1976.
54. De Oliveira F: Pericytes in diabetic retinopathy. *Br J Ophthalmol* 50:134–143, 1966.
55. Hoover R, Rubin R, Wise C, et al: Adhesion of normal and sickle erythrocytes to endothelial monolayer cultures. *Blood* 54:872–875, 1979.
56. Hebbel RP, Yamada O, Moldow CF, et al: Abnormal adherence of sickle erythrocytes to cultured vascular endothelium. *J Clin Invest* 65:154–160, 1980.
57. Wade N: Guillemin and Schally: The years in the wilderness. *Science* 200:279–282, 1978.
58. Wade N: Guillemin and Schally: The three-lap race to Stockholm. *Science* 20:411–415, 1978.
59. Kirber WM, Nichols CW, Grimes PA, et al: A permeability defect of the retinal pigment epithelium: Occurrence in early streptozotocin diabetes. *Arch Ophthalmol* 98:725–728, 1980.
60. Engerman RL: Animal models of diabetic retinopathy. *Trans Am Acad Ophthalmol Otolaryngol* 81:OP710–715, 1976.
61. Engerman RL, Bloodworth JMB Jr, Nelson S: Relationship of microvascular disease in diabetes to metabolic control. *Diabetes* 26:760–769, 1977.
62. Patz A: The role of oxygen in retrolental fibroplasia. *Trans Am Ophthalmol Soc* 66:940–985, 1968.
63. Pickup JC, Keen H, Parsons JA, et al: The use of continuous subcutaneous insulin infusion to achieve normoglycaemia in diabetic patients. *Diabetologia* 13:425, 1977.
64. Tamborlane WV, Sherwin RS, Genel M, et al: Reduction to normal of plasma glucose in juvenile diabetes by subcutaneous administration of insulin with a portable infusion pump. *New Engl J Med* 300:573–578, 1979.
65. Palmberg P, Smith M, Waltman S, et al: The natural history of retinopathy in insulin-dependent juvenile-onset diabetes. *Ophthalmology* 88:613–618, 1981.

2

INFLAMMATORY DISORDERS

INTRODUCTION

INFLAMMATORY DISORDERS of the retina and choroid comprise a large group of highly destructive diseases which are frequently blinding and in certain cases are also very painful. Characterized by the accumulation of inflammatory cells and fluid (edema), these diseases often affect not only the retina and choroid but also the vitreous body and the front of the uvea (the ciliary body and iris). When the iris and ciliary body are involved, secondary glaucoma and cataract related to the inflammation may result. Although all these disorders are commonly referred to as "uveitis," it should be stressed that parts of the eye other than the uvea are frequently involved.

Inflammatory disorders of the retina can cause severe visual impairment or blindness, particularly when they affect the macula, the nerve fibers that lead from the macula to the optic nerve, or the optic nerve itself. Although people with uveitis may retain peripheral vision, they generally will not be able to see more than the large "E" on a visual acuity chart or read ordinary newsprint without the aid of very strong magnifiers. In addition to its blinding effects, inflammation may produce various other symptoms including floating spots, image distortion, severe sensitivity to light, and pain.

According to the National Society to Prevent Blindness, uveitis and chorioretinitis resulted in approximately 1,300 new cases of legal blindness in 1978. Stated in other terms, 2.8 percent of all new cases of registered blindness in the United States in that year were attributable to inflammatory disorders of the retina and uvea. In 1972, an estimated 67,000 Americans had severe visual impairment from uveitis, and 23,000 of these were legally blind. In that same year, an estimated $10 million was spent for the medical care of such patients. These figures probably represent only the tip of the iceberg for ocular disability from inflammatory disorders, because a surprising number of patients slip through routine screening examinations unnoticed. Moreover, a loss of visual acuity in one eye is often not noticed by the patient until the second eye begins to be affected.

Although the major diseases to be discussed in this section are concerned with specific pathology in the retina and choroid, it should be emphasized that the iris and ciliary body are often affected by the same inflammatory process. This is particularly true of those forms of uveitis that are called "diffuse uveitis." They include such entities as ocular sarcoidosis and the Vogt-Koyanagi-Harada syndrome.

Iritis may also occur with such specific chorioretinal inflammations as toxoplasmosis, resulting in the formation of synechias which reduce the ability of the pupil to react to light and may lead to cataract formation as a result of permeability changes that take place in the lens capsule. Iritis may also cause secondary glaucoma by plugging spaces in the trabecular meshwork with fibrin and inflammatory cells. When this glaucoma is acute and severe, it may cause blurred vision and severe pain due to corneal edema.

Inflammatory diseases of the retina and uvea generally are divided into two major categories: infectious or noninfectious. Infectious agents that destroy the retina include viruses, bacteria, fungi, and protozoa. Noninfectious inflammatory diseases of the retina and choroid include autoimmune reactions and other disorders related to the immunologic defense system of the body.

SUBPROGRAM OBJECTIVES

- To establish the fundamental causes and etiologic factors responsible for uveitis.
- To develop improved methods for the diagnosis, therapy, and prevention of uveitis.
- To establish the clinical manifestations, epidemiology, natural course, and prognosis of various forms of uveitis.

OVERVIEW OF CURRENT RESEARCH SUPPORT

The National Eye Institute supported 14 research projects at a total cost of $1,558,000 in FY 1981 dealing with inflammatory disorders of the retina and choroid. Important research in this area is also being conducted intramurally by the National Eye Institute in Bethesda, Maryland. A few related projects are currently funded by other Institutes at the NIH, the Veterans Administration, and by private funding agencies.

NEI-supported research projects cover a wide range of individual studies, including investigations of specific pathogens such as *Toxoplasma gondii* or the herpes viruses; immunologic phenomena such as immune complex disease, autoimmunity to rhodopsin or to soluble antigens derived from the retina (S-antigen); and immunogenetic phenomena that predispose certain individuals to the development of inflammatory diseases.

RECENT ACCOMPLISHMENTS

Diagnosis

Great advances have been made over the last decade in the identification and isolation of infectious agents that cause uveitis. In endophthalmitis caused by bacteria and fungi, direct sampling of the vitreous by either simple aspiration or vitrectomy has facilitated early diagnosis of infection and permitted early initiation of sight-saving therapy.[1] Even when bacterial agents could not be identified, their presence could be inferred by the lowered glucose level of the vitreous humor or the detection of bacterial products such as endotoxins.[2] The analysis of antibodies in the aqueous and vitreous humors has facilitated the diagnosis of nematode worm infections of the uvea.[3] Serologic techniques of great accuracy have been developed for the determination of antibody titers. These include various radioimmunoassays and the enzyme-linked immunosorbent assay (ELISA test) for the detection of antibodies in minute volumes of fluid. The latter technique is being applied to the immunologic diagnosis of many infectious entities including toxoplasmosis.[4] Since the test requires only 10 microliters of fluid, as many as 10 replicate tests can be done on a single sample of aqueous humor.

Ultrasonography and computerized axial tomography have been used to ascertain the location and density of various lesions at the posterior pole of the eye. This is particularly useful when opacities of the lens or vitreous prevent adequate visualization of these structures. These techniques can reveal whether the retina has become detached as a result of an inflammatory insult. They can show the presence of a cyclitic membrane behind the lens of an affected individual, and they can be useful in distinguishing neoplastic lesions from inflammatory lesions.

Fluorescein angiography has greatly facilitated the ability to diagnose the vascular lesions that accompany various inflammatory states of the retina and choroid. Leakage of the dye through the walls of diseased retinal vessels may appear in a very specific pattern in certain inflammatory diseases. The clinical and pathological correlates of these angiographic findings have been established in diseases such as the Vogt-Koyanagi-Harada syndrome[5] and Behcet's disease.[6]

Special enzyme studies performed on either the blood or the aqueous humor allow an inference of the presence of disease in individuals with specific clinical findings. Thus, the angiotensin converting enzyme has been found in the serum of patients with ocular sarcoidosis.[7] The occasional presence of abnormally high levels of lactate dehydrogenase (LDH) in the aqueous humor of patients with retinoblastoma may make it possible to distinguish this disease from nematode endophthalmitis in children.[8] However, some investigators doubt the usefulness of this test, and the potential risk of seeding tumor cells along the track of the needle aspiration must always be borne in mind.

The use of the gallium scan has allowed localization of inflammatory cell infiltrations in the lacrimal glands and mediastinum. These findings, coupled with the presence of elevated levels of angiotensin converting enzyme in the blood, permit a presumptive diagnosis of ocular sarcoidosis even in the absence of the classical features of the clinical syndrome.[9]

Chorioretinal biopsy, initiated in the rabbit[10] and perfected in the dog,[11] eventually may be a practical and relatively safe method of diagnosis in man as

well.[12] In carefully selected cases, this technique may permit a specific light- and electron-microscopic diagnosis of inflammatory lesions affecting the retina and choroid and thereby guide the physician toward the most appropriate modes of therapy. Thus far it has been applied only to the peripheral retina. However, it is likely that postequatorial biopsies, particularly of areas nasal to the optic disc, will eventually become a practical reality. Provided that inflammatory diseases of the retina and choroid have a specific histologic pattern, the opportunity for precise diagnosis of these diseases will be greatly enhanced by direct chorioretinal biopsy. This is particularly true of diseases in which a particular organism can be seen in tissue sections or isolated in culture. The risk of using potentially toxic antimicrobial therapy in such patients would be justified if a precise diagnosis could be made by chorioretinal biopsy.

Understanding the Pathogenesis of Uveitis

Understanding of the immunologic events that accompany certain inflammatory conditions of the retina and choroid has been enormously enhanced by studies of experimental inflammatory eye disease in animal models. Four distinct immunologic mechanisms contributing to inflammation in ocular tissues have been identified. These are: anaphylactoid reactions, cytotoxic reactions, immune complex-mediated reactions, and cell-mediated reactions. Because of the presence of certain markers on the cell membranes of lymphocytes, it is possible to distinguish T-lymphocytes from B-lymphocytes in tissue sections.[13] Thus, certain types of inflammatory reactions can be assigned to specific cells or their mediators. Through the use of radioactively tagged antibodies, the presence of complement in aggregates of immune complexes can be identified in tissue sections. It is thus possible to assign a specific role for immune complexes and for complement to specific diseases such as Behcet's syndrome.[14] Analysis of the activities of macrophages and of their specific enzyme products has greatly facilitated the understanding of diseases such as leprosy or mucocutaneous candidiasis. Both diseases may have tragic effects on the eye, although they are normally well controlled in individuals whose immune systems are not suppressed.

Great strides have been made in the analysis of autoimmune events within the eye. Rhodopsin and S-antigen of mammalian photoreceptors appear capable of stimulating autoimmune reactions under some circumstances.[15] The manner in which these potential antigens become exposed to the lymphoreticular system of the body is not yet understood. Therefore, it is not clear why normal individuals do not develop these autoimmune reactions. The initial site where these autoantigens are processed has not been determined. The pigment epithelium may act as a conventional macrophage in this processing, but it is not known whether the surface membranes of the pigment epithelial cells contain Ia antigens, which are necessary components of antigen-processing cells. These autoimmune reactions may be tied to a defect in immunoregulation. With regard to the latter, subsets of T-lymphocytes, called "helper T-cells" or "suppressor T-cells" respectively, appear to exert a major influence on the development of autoimmune disease. Analysis of the subsets of T-cells in inflamed ocular tissues has not yet been performed, although circulating T-suppressor cells were increased in one study on patients with posterior uveitis.[16] Cyclosporine, an essentially T cell-specific drug,[17] has been shown to prevent S-antigen induced uveitis in the rat.[18]

Treatment

New antiviral substances, bactericidal agents, antiprotozoal substances, antifungal agents, and immunosuppressive drugs have greatly enhanced the ability to cure infections of the retina, choroid, and vitreous.

Bacterial infections of the inner eye (endophthalmitis), which formerly resulted in the total destruction of vision and an atrophic globe, can now be treated effectively by a combination of intravenous, subconjunctival, and intravitreal inoculations of substances such as gentamicin and cephaloridine. Exhaustive studies have been performed on animal eyes to determine which routes of inoculation are most effective for particular antimicrobial agents.[19] Vigorous antimicrobial therapy, combined in some instances with vitrectomy, has saved the eyes of a large number of individuals who sustained infections after a penetrating injury or during intraocular surgery.

Viral infections of the retina such as those caused by the herpes viruses or cytomegalovirus are principally diseases of the newborn. But, adults whose immune systems have been compromised by diseases such as Hodgkin's disease or by immunosuppressive therapy given to retard the rejection of organ transplants may also suffer from cytomegalovirus retinitis. Attempts have been made to treat such infections with adenine arabinoside,[20] transfer factor,[21] or carefully controlled reduction of the dosage of the immunosuppressive agents that they were taking.[22]

Fungal diseases of the retina such as those caused by *Candida albicans* can be treated effectively by combinations of amphotericin B and flucytocine, provided that the diagnosis is promptly made. Once the disease has spread anteriorly into the vitreous, it is usually necessary to perform a total vitrectomy and inject antifungal agents into the vitreous cavity.

New agents that can be given with relative safety by parenteral routes include miconazole and ketoconazole. The efficacy of these agents in the treatment of systemic fungal infections such as coccidioidomycosis has been established. In all these infections, the potential risk from the toxicity of the drug must be carefully balanced against its beneficial effects.

New agents such as clindamycin[23] and minocycline[24] are effective in treating toxoplasmic retinochoroiditis. This disease, caused by a one-celled intracellular parasite, destroys the central vision of thousands of people worldwide every year. Recurrences of the inflammation, attributed to the release of parasites from intraretinal cysts, are the most distressing aspect of the disease. There is now some indication that clindamycin and its derivatives may penetrate the cysts of *Toxoplasma* and inactivate organisms that might otherwise be free to cause recurrent retinal infections.

Autoimmune diseases of the retina and choroid that once had a hopeless prognosis have now been satisfactorily treated by immunosuppressive agents such as chlorambucil and cyclophosphamide. In diseases such as Wegener's granulomatosis, more a corneal problem than a uveal problem, cyclophosphamide has arrested the process in virtually 100 percent of the treated cases.[25] Behcet's disease of the retina, a disorder of almost uniformly bad prognosis, has now been treated very effectively with immunosuppressive agents such as chlorambucil. While all these agents have potentially serious side effects such as the development of malignant neoplasms, sterility, and serious blood dyscrasias, the risk-benefit ratio favors their use in a number of these otherwise hopeless conditions.

In addition, a clinical trial was recently begun at the NEI to determine whether Cyclosporine is helpful to posterior uveitis patients with demonstrated sensitivity to S-antigen.

Surgery for a number of forms of uveitis has also proved to be of great benefit. In the past, severe inflammatory reactions in the vitreous often caused traction detachments of the retina. They also produced dense membranes that interrupted the passage of light rays to the central portion of the retina. The development of vitrectomy has saved useful vision for a large number of patients. This form of therapy has, for example, proved effective in the treatment of severe, chronic vitreous inflammations resulting from toxoplasmic retinochoroiditis.[26] Severe endophthalmitis of either bacterial or fungal origin has also been treated effectively by vitrectomy.[27]

Other important forms of therapy being evaluated include the use of photocoagulation for treating indolent toxoplasmic infections of the retina[28] and eradicating subretinal neovascular networks such as those that appear in the wake of histoplasmic choroiditis. These treatments, added to a long list of antimicrobial and anti-inflammatory regimens, have added greatly to the ability to control severe inflammatory lesions of the retina and choroid.

RESEARCH NEEDS AND OPPORTUNITIES

Improved Diagnosis: Identification of Pathogens

Because the precise etiology of the majority of uveitis cases remains unknown, new efforts must be directed toward the development of methods for making a direct diagnosis from ocular tissues. Important beginnings have been made by the isolation of bacteria and fungi from vitreous aspirates, obtained by direct sampling from eyes afflicted with endophthalmitis. These studies should now be extended to include viruses and other agents, which have proved more difficult to isolate.

New technological methods are available, including co-cultivation of tissue specimens in special tissue culture media that utilize specific cell lines. Isolation of suspected viruses might also be attempted in immunosuppressed mice. When abundant vitreous exudate is present, attempts should be made first to isolate the viruses from the vitreous itself. When the locus of the inflammation is limited to the retina, it may be possible to isolate the positive agents from peripheral chorioretinal biopsies.

Analysis of Immunologic Events in Diseased Retinal Tissue

There is an urgent need to analyze the cellular and humoral components of inflammatory disease in specimens of human ocular tissue. Rapidly expanding technology in this area recently has provided methods for identifying cell-membrane markers, immune complex depositions, complement deposition, and other immunologic factors that contribute to the inflammatory process. Different forms of uveitis may be distinguishable on the basis of the immunologic components participating in a given reaction. For example, the deposition of immune complexes may be a major factor in the pathogenesis of Behcet's disease; this has been demonstrated in the lung and skin,[14] but ocular tissue has not yet been extensively examined for evidence of these phenomena. On the other hand, cell-mediated reactions may play a much more important role in diseases such as ocular toxoplasmosis and cytomegalovirus infections of the retina.

The rapidly expanding field of immunology is likely to make the most significant contributions to research into the causes of uveitis. Particularly

within the last decade, the application of basic immunologic laboratory techniques to the study of ocular tissues has yielded highly important information, providing the researcher and clinician with new insights into the probable cause of various forms of uveitis. For example, a recent study[29] showed that patients with recurrent ocular toxoplasmosis manifested high levels of cellular reactivity to S-antigen, a soluble autoantigen derived from the outer segments of the retinal photoreceptors. It seems likely that toxoplasmic infection caused destructive changes in the photoreceptors, which allowed for presentation of this autoantigen to the body's lymphoreticular system. Recurrent lesions of toxoplasmic retinochoroiditis may represent an immunologic reaction not only to *Toxoplasma* antigens, but to autoantigens of the retina as well. Without the development of tissue culture techniques that have permitted the culture of lymphocytes from the buffy coat of the patient's blood in the presence of antigens, such studies would not have been possible.

Defects in immune regulation need to be investigated in ocular inflammatory disease and other forms of autoimmune disease. It seems clear that subsets of T-lymphocytes called helper cells or suppressor cells modulate immunologic processes at all times and in all tissues. Defects in this system give rise to autoimmune diseases such as lupus erythematosus. However, these phenomena have not yet been studied in the eye. Study of these factors is likely to provide crucial information concerning the pathogenesis of such diseases as sympathetic ophthalmia, lupus erythematosus, and other forms of retinal vasculitis.

Analysis of Genetic Factors in Inflammatory Disease

Increased effort should be devoted to the analysis of genetic factors that may predispose certain individuals to the development of specific types of inflammatory disease. Preliminary research, mainly by Japanese investigators, showed a link between the development of Behcet's disease and the possession of the HLA antigen B-5. Although this research has been partially substantiated by findings in French and Turkish patients,[30] it does not seem to apply to the majority of Caucasian patients with this disease. The same is true of patients with the Vogt-Koyanagi-Harada syndrome which is associated with the HLA antigen BW22-J in Japan; this finding has not been confirmed in studies of Caucasian patients.

It seems likely that the combined activity of certain "B" and "D" locus antigens may ultimately control the way in which certain immunologically mediated diseases are expressed. Because the determination of "D" antigens is difficult and expensive, this line of research has not been pursued actively in inflammatory eye diseases. However, this is an area that must be pursued, for it is important to determine which patients are at risk for developing certain kinds of inflammatory disease of the posterior segment. Careful monitoring of the patients at risk may enable ophthalmologists to detect such disease at its earliest stages. Effective therapy can often be delivered at these stages, whereas advanced cicatricial disease is difficult to deal with by any mode of currently available therapy.

Establishment of Animal Models of Retinal Inflammatory Disease

Human inflammatory diseases of the posterior segment are often mimicked by certain spontaneously occurring diseases in animals. For example, a species of dog called the Akita develops a form of diffuse uveitis which is similar to the Vogt-Koyanagi-Harada syndrome. It produces bilateral iriodocyclitis, serous detachments of the retina, vitiligo, and poliosis. The disease seems to be specific for this particular breed of dog. Genetic studies as well as histopathologic and immunological studies need to be conducted on such animals to determine the relationship of this disease to human disease.

Uveitis may be produced experimentally by a number of standard procedures such as the injection of bland serum proteins into the vitreous. However, most experimentation in this field has been done in outbred species such as the rabbit. Such experiments should be conducted in inbred species of known genetic background such as certain mice and guinea pigs, which are available for this purpose. Only by the use of inbred species can immunopathological events be interpreted accurately. This is an important area of research that requires further development.

Ultimately, experimental uveitis that closely simulates naturally occurring conditions in man must be produced in the retinas of subhuman primate species. To draw valid conclusions about the parallelism between experimentally produced disease and naturally occurring human disease, lesions should be produced in retinal tissue in which the anatomy and vascular supply can be compared to that of man.

Utilization of Clinicopathologic Correlations of Inflammatory Eye Disease in Man

Systematic examination is needed of enucleated or autopsy eyes from individuals whose inflammatory eye disease has been well-documented. The history of the disease and its physical manifestations, fundus photography, fluorescein angiography, field studies, and electrophysiologic data should be assembled according to carefully prepared protocols. Special

attempts should then be made to obtain these eyes at the time of enucleation or upon the death of the patient. Correlation of histopathologic, ultrastructural, immunological, and biochemical studies with clinical findings should be made in a search for pathogenetic mechanisms.

Analysis of the biochemical events that lead to and accompany inflammation has resulted in a great deal of information that is potentially applicable to the study of ocular inflammation. Of particular interest are chemotactic substances such as "eosinophil chemotactic factor" (which is released by degranulating mast cells), leukotactic molecules such as the C3B component of complement, and lymphokines secreted by sensitized T-lymphocytes upon contact with a specific antigen. The latter substances are responsible for the arrest of macrophages at tissue sites where they are needed to combat infection and for agglutinating macrophages under certain circumstances. These same macrophages may eventually liberate "suppressor factors" (probably prostaglandins), which play a role in turning off inflammation; this is part of the body's mechanism for maintaining homeostasis. Inflammation plays a useful role in the body, but it must always be kept from getting out of hand.

Even the small number of biochemical substances mentioned in the paragraph above suggest the complexity of the chemical events that occur simultaneously in inflamed tissues. Methods have been devised to measure these substances, and in most cases microanalytical methods suitable for the study of small samples of ocular tissue are already available. These techniques need to be applied to studies of the eye if an intelligent analysis of the events leading to ocular inflammation is to be made.

In this connection, the National Eye Institute recently sponsored a series of three workshops concerned with ocular immunology. The first of these dealt with immunogenetics and transplantation immunity; the second, with autoimmunity; and the third, with infection and inflammation. At these meetings, the state of the art in each of these fields was presented by experts representing a wide range of investigatory efforts. Clearly, the technology, which was developed mainly in fields outside of ophthalmology, is available, but how can the manpower be obtained to apply these highly sophisticated techniques to the problems of ocular inflammation? If experts highly experienced in these techniques can be attracted to vision research, can they be supported on a long-term basis? Can physicians who are trained as ophthalmologists acquire the necessary laboratory skills to study these problems? Can these ophthalmologists be supported? These are important questions that as yet have not been answered.

Application of Immunologic Studies to Other Related Diseases of the Posterior Segment

Recent studies suggest that the immune system may play some role in certain degenerative diseases of the retina.[31] In retinitis pigmentosa, for example, there is some indication of altered reactivity to S-antigen. It is highly likely that this has a genetic basis. Further studies need to be done on the possible immunopathogenesis of retinitis pigmentosa and related degenerative diseases. It is conceivable that if immunoregulatory mechanisms are defective in some of these patients, the defects might be corrected therapeutically, but this is a long-range objective.

The epidemiology of many theoretically preventable diseases of the retina and choroid has not been satisfactorily investigated. This is particularly true of infectious entities such as toxoplasmosis and toxocariasis, in which the exact mode of man's acquisition of the disease has not been established. Surveys such as that recently published by Schantz et al[32] have identified risk factors for toxocaral ocular larva migrans in man, particularly with respect to exposure to dogs and cats, but no systematic analysis of the factors that predispose an infected person to inflammatory disease of the retina and choroid has been undertaken. Clearly, this is a very large task, since the factors contributing to the appearance of inflammatory disease in certain individuals, but not others, must be multiple. Endogenous factors (genetic background, endocrine status, immunologic status, age, sex, and nutritional status), environmental factors (climate, exposure to vectors, and industrial conditions), and lifestyle (eating and sexual habits) must all be taken into consideration. Epidemiologic methods for the analysis of these factors, aided by computerized logging and sorting of the data, are currently available and have been utilized in the study of other disorders. Such methods also should be employed in an epidemiologic study of uveitis.

Exploration of New Forms of Immunosuppressive Therapy

Great advances have been made in the use of immunosuppressive therapy for the control of intractable idiopathic uveitis. However, the results of these therapies have not been analyzed by randomized double-masked trials. Since the number of patients is usually small at any one institution, collaborative studies involving multiple institutions could properly evaluate the efficacy and safety of any one drug or combination of drugs. Satisfactory protocols must be developed so that accurate conclusions can be drawn from the results.

Training Needs

Sufficient numbers of research-oriented personnel must be trained in modern methods concerned with infectious and immunologic diseases of the eye. Such personnel must come from the basic sciences and from ophthalmology and be trained in institutions where large numbers of patients with inflammatory diseases of the retina and choroid are seen on a regular basis. Adequate equipment must be available to document the physical aspects of the inflammatory eye disease, and the neurophysiologic changes that may have affected the ocular tissues. The primary training mechanism should be postdoctoral fellowships granted to individuals who show promise of doing creditable work in academic research.

Additional educational opportunities should be made available through NEI-sponsored workshops and symposia dealing with infectious and inflammatory diseases of the eye, scheduled to take maximal advantage of emerging research methodologies. The agenda of the workshops should be such that scientific interactions can occur between researchers in ophthalmology and those in various basic sciences.

RECOMMENDATIONS

Based on the foregoing assessment of recent accomplishments, current activities, and research needs and opportunities in "Inflammatory Disorders," the Panel has made the following recommendations concerning research in this subprogram over the next five years. These have been grouped under two headings: Program Base and Program Development Priorities.

The Program Base includes areas of ongoing research where the current level of activity is considered adequate, or areas of ongoing research in which there may be great need for additional activity, but where, in the Panel's judgment, little or no opportunity (new methods or insights) exists at present to justify a significant expansion of effort. Nonetheless, additional applications for research grants in these areas may be funded if they are innovative and of very high quality as determined by the NIH peer review system.

Program Development Priorities include areas of ongoing research in which new knowledge and techniques offer particular opportunities for scientific progress, or promising new areas of research in which there is little or no support at present but where there is both great need and high potential for success. Such areas are judged to warrant significantly increased support over the next five years, provided that high quality applications for research grants in these areas are forthcoming.

Program Base

- Develop and improve methods for the diagnosis of specific inflammatory diseases of the retina and choroid.
- Utilize inbred strains of animals to produce models of chronic or recurring inflammatory diseases of the retina and choroid; immunogens that can be standardized should be used.
- Determine the epidemiology, pathogenesis, and nature of the uveal response to infectious agents.
- Analyze the immunopathogenic responses in uveitis.
- Develop drug delivery systems that optimize the delivery of pharmacological agents to target sites in the retina or uvea.

Program Development Priorities

- Search for the etiology of various inflammatory diseases caused by infectious agents using the most modern methods for isolating the pathogen.
- Establish the usefulness and safety of vitreous and chorioretinal biopsies for diagnosing and managing inflammatory disorders.
- Make maximum use of surgical specimens and autopsy eyes to obtain information on the pathogenetic mechanisms responsible for inflammatory diseases in humans.
- Determine the role of autoantigens, particularly those derived from the retina, in the induction of chronic inflammatory disease.
- Conduct randomized controlled clinical trials of antimicrobial and anti-inflammatory agents in the treatment of retinal and uveal inflammatory diseases.

RESOURCE REQUIREMENTS

After reviewing current research grant support in each of these categories and assessing the need and potential for future development, the Panel has estimated the number of projects it believes are needed to carry out its recommendations in FY 1983. These estimates are shown in the table on the following page. For a discussion of the general basis and significance of these projections, see the "Summary" at the beginning of this report.

RESOURCE TABLE

INFLAMMATORY DISORDERS

	No. of Grants FY 1981	Panel Recommendation FY 83	
		Add. Grants	Total Grants
Program Base			
A. Develop and improve methods for the diagnosis of specific inflammatory diseases.	1	1	2
B. Utilize inbred strains of animals to produce models of chronic or recurring inflammatory diseases.	3	0	3
C. Determine the epidemiology, pathogenesis, and nature of the uveal response to infectious agents.	4	0	4
D. Analyze the immunopathogenic responses in uveitis.	5	0	5
E. Develop drug delivery systems that optimize the delivery of pharmacological agents to target sites in the retina or uvea.	0	1	1
Program Development Priorities			
A. Search for the etiology of various inflammatory diseases caused by infectious agents.	0	1	1
B. Establish the usefulness and safety of vitreous and chorioretinal biopsies.	0	1	1
C. Make maximum use of surgical specimens and autopsy eyes for information on pathogenetic mechanisms.	0	2	2
D. Determine role of autoantigens in the induction of chronic inflammatory disease.	1	3	4
E. Conduct randomized controlled clinical trials of antimicrobial and anti-inflammatory agents.	0	2	2
Subtotal Grants	**14**	**11**	**25**
(% of Program)	**(4)**	**(10)**	**(5)**
Total Estimated Cost	**$1,558,000**	**$1,067,000**	**$2,625,000**

REFERENCES

1. Forster RK: Endophthalmitis: Diagnostic cultures and visual results. *Arch Ophthalmol* 92:387–392, 1974.
2. McBeath J, Forster RK, Rebell G: Diagnostic limulus lysate assay for endophthalmitis and keratitis. *Arch Ophthalmol* 96:1265–1267, 1978.
3. Biglan AW, Glickman LT, Lobes LA: Serum and vitreous *Toxocara* antibodies in nematode endophthalmitis. *Am J Ophthalmol* 88:898–901, 1979.
4. Lin TM, Halbert SP, O'Connor GR: Standardized quantitative enzyme-linked immunoassay for antibodies to *Toxoplasma gondii*. *J Clin Microbiol* 11:675–681, 1980.
5. Manor RS: Particular aspects of the Vogt-Koyanagi-Harada Syndrome. *Ophthalmologica* 165:425–427, 1972.
6. Shimizu K: Fluorescein fundus angiography in Behcet's syndrome. *Nippon Ganka Gakkai Zasshi* 74:1432–1448, 1970.
7. Weinreb RN, Kimura SJ: Uveitis associated with sarcoidosis and angiotensin converting enzyme. *Am J Ophthalmol* 89:180–185, 1980.
8. Shields JA, Lerner HA, Felberg NT: Aqueous cytology and enzymes in nematode endophthalmitis. *Am J Ophthalmol* 84:319–322, 1977.
9. Nosal A, Schleissner LA, Mishkin FS, et al: Angiotensin-I converting enzyme and gallium scan in non-invasive evaluation of sarcoidosis. *Ann Intern Med* 90:328–331, 1979.
10. Peyman GA, Meisels HI, Batko KA, et al: Full thickness eye-wall biopsy: I. An experimental approach to the tissue diagnosis and study of choroidal and retinal lesions. *Invest Ophthalmol Vis Sci* 14:484–487, 1975.
11. Constable IJ, Slatter DH, Horne R: Chorioretinal biopsy in dogs. *Invest Ophthalmol Vis Sci* 19:603–609, 1980.
12. Constable IJ: Problems of chorioretinal biopsy. *J R Soc Med* 73:408–413, 1980.
13. Knowles DM, Holck S: Tissue localization of T-lymphocytes by the histochemical demonstration of acid alpha-naphthyl acetate esterase. *Lab Invest* 30:70–76, 1978.
14. Maciejewski W, Bandmann HF: Immune complex vasculitis in a patient with Behcet's disease. *Arch Dermatol* 264:253–256, 1979.
15. Wacker WB, Donoso LA, Kalsow CM, et al: Experimental allergic uveitis: Isolation, characterization, and localization of a soluble uveitopathogenic-antigen from bovine retina. *J Immunol* 119:1949–1958, 1977.
16. Nussenblatt RB, Cevario SJ, Gery I: Altered suppressor-cell activities in uveitis. *Lancet* 2:722–724, 1980.
17. Brent L: Cyclosporin A: A discussion of its clinical and biological attributes—a summary of a workshop. *Transplant Proc* 12:234–238, 1980.
18. Nussenblatt RB, Rodrigues MM, Wacker WB, et al: Cyclosporin A: Inhibition of experimental autoimmune uveitis in Lewis rats. *J Clin Invest* 67:1228–1231, 1981.
19. Barza M, Kane A, Baum J: Oxacillin for bacterial endophthalmitis: Subconjunctival, intravenous, both, or neither? *Invest Ophthalmol Vis Sci* 19:1348–1354, 1980.
20. Rytel MW, Kauffman HM: Clinical efficiency of adenine arabinoside in therapy of cytomegalovirus infections in renal allograft patients. *J Infect Dis* 133:202–205, 1976.
21. Rytel MW, Aaberg TM, Dee TH, et al: Therapy of cytomegalovirus retinitis with transfer factor. *Cell Immunol* 19:8–21, 1975.
22. O'Connor GR: Uveitis and the immunologically compromised host. *New Engl J Med* 299:130–132, 1978.
23. Tabbara KF, O'Connor GR: Treatment of ocular toxoplasmosis with clindamycin and sulfadiazine. *Ophthalmology* 87:129–134, 1980.
24. Tabbara KF, Sakuragi S, O'Connor GR: Minocycline in the chemotherapy of murine toxoplasmosis. *Parasitology* 84:297–302, 1982.
25. Fauci AS, Katz P, Haynes BF, et al: Cyclophosphamide therapy of severe systemic necrotizing vasculitis. *New Engl J Med* 301:235–238, 1979.
26. Fitzgerald CR: Pars plana vitrectomy for vitreous opacity secondary to presumed toxoplasmosis. *Arch Ophthalmol* 98:321–323, 1980.
27. Hanscom TM, Maxwell A: Corynebacterium endophthalmitis: Laboratory studies and report of a case treated by vitrectomy. *Arch Ophthalmol* 97:500–502, 1979.
28. Ghartey KN, Brockhurst RJ: Photocoagulation of active toxoplasmic retinochoroiditis. *Am J Ophthalmol* 89:858–864, 1980.
29. Nussenblatt RB, Geri I, Ballintine EJ, et al: Cellular immune responsiveness of uveitis patients to retinal S-antigen. *Am J Ophthalmol* 89:173–179, 1980.
30. Yazici H, Chamberlain MA, Schreuder I, et al: HLA antigens in Behcet's disease: A reappraisal by a comparative study of Turkish and British patients. *Ann Rheum Dis* 39:344–348, 1980.
31. Brinkman CJJ, Pinckers AJLG, Broekhuyse RM: Immune reactivity to different retinal antigens in patients suffering from retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 19:743–750, 1980.
32. Schantz PM, Meyer D, Glickman LT: Clinical, serologic, and epidemiologic characteristics of ocular toxocariasis. *Am J Trop Med Hyg* 28:24–28, 1979.

3

TUMORS

INTRODUCTION

THE TWO MOST frequent intraocular tumors are choroidal melanoma and retinoblastoma. Because they threaten life as well as sight, early detection and effective treatment are of particular concern to the ophthalmologist. The development of improved therapies for these tumors requires a better understanding of their basic biology and, for choroidal melanoma, a clearer understanding of its natural course.

Ocular Melanoma

Malignant melanomas of the choroid and ciliary body are the most common primary intraocular neoplasms, comprising approximately 80 percent of all eye malignancies. The annual age-adjusted incidence of choroidal melanoma is 0.7 per 100,000 population in the United States,[1] and approximately 1,500 new cases are diagnosed annually. The overall mortality rate for these tumors at five years is 35 percent, but for large or cytologically malignant tumors (that is, epithelioid cell type), the five-year mortality rate approaches 90 percent.[2]

Knowledge of the epidemiology and pathogenesis of these tumors is limited. Age and race are recognized risk factors for the development of choroidal melanoma. Although sunlight exposure is cited as a major causative factor for melanomas of the skin and probably the conjunctiva, the incidence of choroidal melanoma shows no such relationship.[1,3]

Diagnosis of ciliary body and choroidal melanomas has become highly accurate at eye centers where experienced clinicians and ancillary testing facilities are available (Figure 1). The rate of mistaken diagnosis, including both false positive and false negative conclusions, is approximately 6 percent.[4] Although the cornerstone of diagnosis in posterior uveal melanoma remains clinical examination by experienced observers, ancillary diagnostic testing, including ophthalmic ultrasound, and fluorescein angiography can be extremely valuable.[5-7] The development of immunologic testing shows some potential for future use in the diagnosis of ocular melanomas, although at present it does not offer results as reliable as other methods.[8-10]

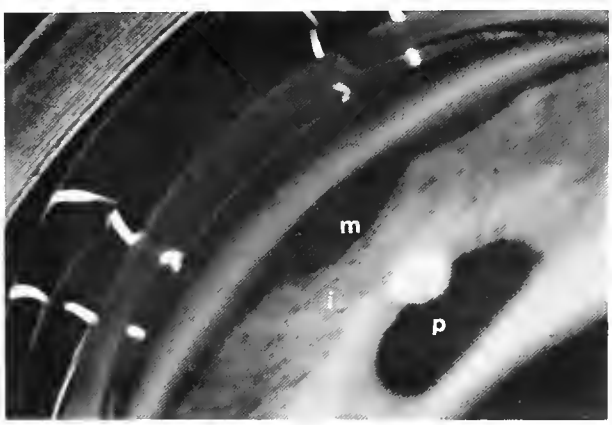


FIGURE 1. A melanoma of the ciliary body (m) invading the angle and spreading onto the surface of the iris (i) as viewed through a special diagnostic gonioscopic contact lens; (p = pupil).

Knowledge of the natural course of ocular melanomas is essential for designing and evaluating treatment regimens. But, limited data are available on the growth characteristics and metastatic potential of both large and small tumors. Virtually nothing is known about the frequency of spontaneous regression of these lesions, and only limited information is available about the frequency of metastatic disease at the time these patients are first seen in the clinic.

Because of these many uncertainties, the efficacy of the standard therapy for melanomas of the choroid, namely, enucleation, in the prevention of metastatic disease has become the focus of intense controversy.[11,12] Alternative management approaches include observation of lesions for evidence of growth, proton beam-irradiation, cryotherapy, laser photocoagulation, and full thickness eye wall resection with tumor removal.[13–16] The relative efficacy of these procedures in preventing or delaying metastatic disease when compared to enucleation has not been established. Thus, the clinician caring for a patient with a choroidal melanoma, particularly a small- or medium-sized lesion in an eye with good visual acuity, faces a difficult dilemma.

Retinoblastoma

Retinoblastoma is the most common intraocular tumor in children and may be the most common congenital tumor of any type. Various studies in the United States and Western Europe in the last 40 years have indicated that retinoblastoma occurs in approximately 1 in 23,000 live births.[17] Not only does retinoblastoma cause blindness, it also can cause death. Moreover, approximately 35 to 40 percent of these cases are hereditary, and other siblings also are at risk. A disturbing feature of retinoblastoma is that its frequency may be increasing.[18]

The incidence of retinoblastoma may be higher in some races and in other parts of the world than the United States and Europe. In a study in Haiti, for example, the estimated frequency of retinoblastoma was calculated at 1 in every 3,300 live births, and the estimated incidence rate was 11 per 100,000.[19] In Nigeria, retinoblastoma was reported to occur in 1 of every 192 patients examined over a two and one-half year period,[20] and in Jamaica its annual incidence was 24 per 100,000 in the 0 to 4 year age group.[21]

An epidemiologic review of 269 deaths secondary to retinoblastoma showed that peak mortality occurred at 2 to 3 years of age and was two and one-half times higher in blacks than whites.[22] In addition, a higher mutation rate for retinoblastoma was reported in blacks than for whites in Ohio.[23]

The costs of this disorder to the individual and society are high. Although retinoblastoma can be accurately diagnosed and effectively treated in about 90 percent of patients, treatment usually is destructive and results in loss of vision (Figure 2). Retinoblastoma victims whose tumors have been cured but whose vision has been lost experience the same economic difficulties and social tragedies as do other victims of childhood blindness.[24] Retinoblastoma patients constitute a significant portion of the population in schools for the blind.

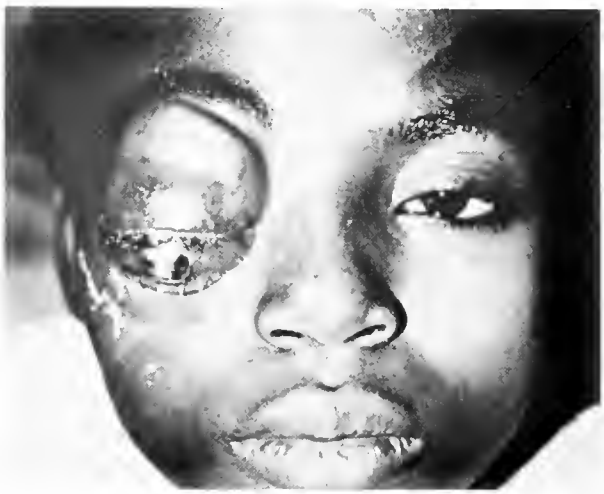


FIGURE 2. Advanced retinoblastoma destroying the eye and invading the surrounding tissues in a child.

The management of retinoblastoma may involve the use of various modalities: two types that treat the entire retina, such as radiotherapy and chemotherapy,[25] and four that treat well localized areas within the retina; these are diathermy, radon seeds and Co^{60} plaques, light coagulation, and cryotherapy.

Some insight has been gained into the pattern of inheritance of retinoblastoma, and in certain clear-cut cases it can be established whether the disease is inherited or familial. On the other hand, it is often difficult to distinguish the hereditary from the nonhereditary form, and it may not be possible to predict which family members will be affected and, if affected, which will have unilateral or bilateral disease.

On a more basic level, the cause of retinoblastoma is unknown, and its pathogenesis is poorly understood. For example, the cell of origin is still controversial. This, of course, has practical significance in determining whether the pathogenesis might lead to diagnostic clues for earlier and more definitive detection. Finally, although retinoblastoma undergoes spontaneous regression more commonly than does any other tumor, nothing is known of the factors that induce the regression.

SUBPROGRAM OBJECTIVES

Ocular Melanoma

- To determine the natural history and pathogenesis of ocular melanoma with special emphasis on utilizing the latest biochemical, histochemical, and immunologic techniques.
- To define the epidemiology of ocular tumors with an emphasis on identifying possible risk factors for their development.
- To determine the efficacy of treatments in randomized controlled clinical trials.
- To understand the role of immunity in the disease process.
- To develop and refine methods of diagnosing and characterizing ocular tumors.

Retinoblastoma

- To determine the etiology and pathogenesis of retinoblastoma.
- To differentiate retinoblastomas that are genetic in origin from those that are not.
- To improve the effectiveness of retinoblastoma treatment with emphasis on preserving vision.

OVERVIEW OF CURRENT RESEARCH SUPPORT

In fiscal year 1981, 15 research grants for the study of ocular melanoma were funded by the National Eye Institute. Areas of investigation included immunodiagnosis, correlation of malignant potential to histopathologic diagnosis, experimental chemotherapy, and a virus-induced ocular melanoma model in the cat. Other studies included investigation of the prognosis, diagnosis, treatment, pathogenesis, and etiology of both retinoblastoma and melanoma.

Three grants were funded in FY 1981 for the study of retinoblastoma. Biochemical and cytogenetic markers, in vitro studies of established cell lines, and animal models in the rat and mouse were the foci of these investigations.

The total cost of these eighteen grants for tumor research was $1,887,000.

RECENT ACCOMPLISHMENTS

Ocular Melanoma

Among the most important advances in understanding the pathogenesis and treatment of choroidal melanoma are: apparent promise for successful treatment with proton beam-irradiation;[14,26] demonstration of an increased incidence of choroidal melanoma in a single population of chemical workers;[27] successful heterotransplantation of choroidal melanoma into the athymic nude mouse;[28] demonstration of tumor specific immune responsiveness in patients with choroidal melanoma;[8,9] chemical induction of uveal melanoma and other intraocular tumors in laboratory animals;[29] and induction of uveal melanomas in cats using the feline sarcoma virus.[30]

Retinoblastoma

Histopathology and Ultrastructure. Fleurettes (Figure 3), structures interpreted to represent photoreceptor differentiation, have been described in retinoblastoma and their implications for prognosis and pathogenesis studied.[31] In addition, the Flexner-Wintersteiner rosette (Figure 4), long the characteristic histopathologic structure of retinoblastoma, has been defined as containing: (1) terminal bars of the luminal limiting membrane, (2) cytoplasmic microtubules, (3) cilia of the 9+0 configuration, (4) laminated membranous structures in the lumen, and (5) acid mucopolysaccharides resistant to hyaluronidase in the lumen. In addition, zonula adherens-like intercellular attachments, macula adherens triple membrane structures, 9+0 cilia, and microtubules most abundant in the Golgi area are characteristic findings in retinoblastoma cells.[19] Other characteristic structures of retinoblastoma included bristle coated vesicles, dense core secretory granules, annulate lamellae,[32,33] and basophilia in areas of blood vessels, characterized as deposits of DNA.[34]

Genetic and Chromosomal Studies. A major advance in understanding the genetics of retinoblastoma has been Knudson's hypothesis suggesting that retinoblastoma is a form of cancer caused by two mutational events.[35] The theory proposes that in the hereditary or prezygotic form, the first mutation is germinal and the second is somatic; in the sporadic (nonhereditary) or postzygotic form, both mutations are somatic.

Further insight into chromosomal changes associated with retinoblastoma has been gained by studying the D-deletion syndrome. Thus far, 13 of 45 patients with this syndrome have been reported to

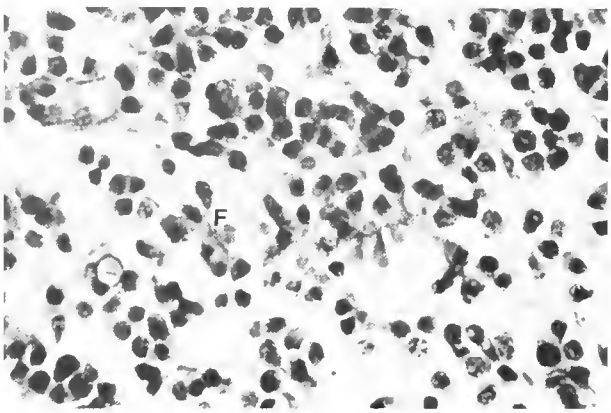


FIGURE 3. Microscopic appearance of retinoblastoma showing fleurettes (f), structures in which the tumor is attempting to make normal retina photoreceptor cells.

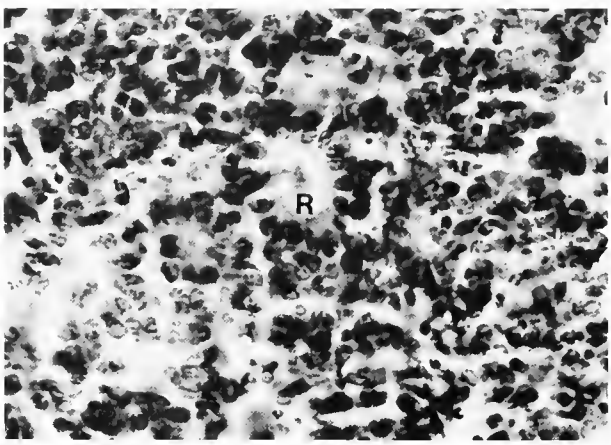


FIGURE 4. The appearance of a retinoblastoma under the microscope showing a characteristic rosette-like pattern (R), which is helpful in diagnosis. (Hematoxylin and eosin stain, 400x magnification.)

have retinoblastoma.[17] With refined techniques, including autoradiography, quinacrine fluorescence, and analysis of the DNA d-r Giemsa pattern, the involved area on the D chromosome in affected patients has consistently been the long arm of chromosome 13. The association of retinoblastoma with the syndrome involving a deletion of this particular chromosome might suggest the absence of the locus that normally suppresses a retinoblastoma gene, or the unmasking of some mechanism that may allow increased susceptibility to tumor development.

Development of Second Tumors in Retinoblastoma Patients. In the past several years, it has been recognized that patients with the bilateral, presumably genetic, form of retinoblastoma have a high incidence of second primary malignancies, and further, that this may occur in patients not receiving radiation.[36] Recently, exciting work has indicated a possible DNA repair deficiency in these patients, probably analogous to the well-described DNA repair defect seen with ultraviolet light in xeroderma pigmentosa patients.[37]

New Techniques for Diagnosis. Ultrasound has proved valuable in confirming the presence of calcification, a characteristic finding in retinoblastoma.[38] Considerable attention has also been given to the measurement of lactic acid dehydrogenase (LDH) levels in serum and aqueous humor in the diagnosis of retinoblastoma. Although serum LDH levels currently are not thought to be predictive of disease activity, and aqueous-to-serum ratios and isoenzyme patterns do not appear to differ significantly from other nonmalignant conditions, total aqueous LDH levels may have clinical significance[39] and prognostic value.[40] Other factors, including carcinoembryonic antigen, alpha fetoprotein, and vanillylmandelic acid (VMA) and monovanillic acid have been considered. However, the results do not lend themselves to routine clinical application.

In Vitro Studies of Human Retinoblastoma. The development of in vitro established cell lines of retinoblastoma[41,42] has made a continuous supply of retinoblastoma cells available, which is widely utilized by investigators throughout the world. Recent studies of these cell lines have demonstrated cellular retinol and retinoic acid binding proteins.[43–47] These in vitro investigations may prove useful in determining the cellular origin of retinoblastoma[48] and give clues for its chemotherapeutic management.[49]

Animal Models of Retinoblastoma. Murine retinoblastoma induced by adenovirus[50] and retinoblastoma in cats induced by the naturally occurring feline sarcomaleukemia virus[51] are available.

RESEARCH NEEDS AND OPPORTUNITIES

Ocular Melanoma

Recognizing the need to define research priorities in this field, the National Advisory Eye Council recommended in 1980 that the NEI convene an Ocular Melanoma Task Force. Such a Task Force

was established in that year to evaluate the problems associated with this disease and recommend what research is needed to solve them.[52] In particular, the Task Force was charged with evaluating conflicting conclusions and recommendations appearing in published reports about the management of ocular melanoma. Research needs and opportunities were identified in six areas—epidemiology, diagnosis, pathology, natural history, therapy, and cell biology. These and other areas are reviewed in the following discussion. Epidemiologic and natural course studies, in particular, were identified by the Task Force as one of the first steps toward the goal of determining how best to manage ocular melanoma.

Epidemiology

Understanding of the epidemiology of choroidal melanoma remains incomplete. In large measure, such studies have focused on ocular findings; data regarding risk factors other than sex, age, and race are usually omitted.[53,54] Potential risk factors that must be studied include: ocular and cutaneous pigmentation; exposure to light; presence of cutaneous nevi, melanoma, or other lesions; possible influence of hormonal factors (for instance, during pregnancy); neoplasms at organ sites other than the skin; occupation; known exposure to potential carcinogens; and sociodemographic factors. These factors can be compared to those already identified as risks for cutaneous melanoma. Epidemiologic case-control studies can identify factors associated with the incidence of uveal melanomas. Patients for such studies could be identified by a national prospective study of patients with uveal melanoma.

Diagnosis and Histopathology

The most important need in diagnosis is the development of reliable standards for differentiating small melanomas from choroidal nevi (nonmalignant). The identification of clinical signs that herald tumor growth would also be of considerable value. Studies are needed that correlate data from various diagnostic studies (such as ultrasonography, wide-angle fundus photography, fluorescein angiography, P^{32}, and immunodiagnostic assays) with clinical course and histopathology. Moreover, standard criteria for the classification and evaluation of data from diagnostic modalities need to be developed.

The development of metastatic uveal melanoma has not been followed in a uniform and longitudinal fashion in a large group of patients. Standards need to be established for medical evaluation of melanoma patients at the time they are first seen in the clinic and at subsequent follow-up examinations for the detection of metastatic disease. These standards should encompass physical examination, laboratory assays (including liver function studies), and radiologic studies such as radioisotope liver scanning or computerized body tomography.

There is considerable divergence among ophthalmologic investigators in the application of the Callender classification to the cytologic study of choroidal melanomas.[55] Cell type has been well recognized as an important prognostic indicator in patients with these lesions who undergo enucleation, yet there is considerable evidence that criteria for cell type are not uniformly applied. A major effort to develop precise, consistent, and reproducible histologic criteria for the description and comparison of ocular melanoms needs to be undertaken. Of foremost concern are: (1) the definition of a uveal nevus, including a consideration of the variations in cell types of different nevi, (2) definition of a uveal melanoma (for example, as compared with a "melanocytic tumor"), (3) adequate term(s) for the description of lesions in the grey zone between nevus and melanoma (for example, terminology for a pure spindle A "melanoma"), (4) strict criteria to provide consistency and uniformity in the use of the Callender classification and in determining the cellular criteria necessary to constitute spindle A, B, mixed and epithelioid cells (for example, the number of epithelioid cells per unit area to constitute a mixed cell lesion), (5) criteria for specifying degree of pigmentation, (6) criteria to designate degree of inflammation, (7) standard terminology to define necrosis, (8) procedures for obtaining reproducible measurements of tumor size in gross specimens and on histopathologic sections, and (9) a standardized method for preparing tumor-bearing eyes for histologic examination.

Natural History

It is important to know how events in the natural history of the tumor are reflected in the histopathology and how such findings correlate with prognosis. The data needed include: (1) an estimation of the amount of pigmentation and number of melanocytes in affected eyes; (2) documentation of presence or absence of preexisting nevi or other precursor lesions; (3) specification of cell type in small melanomas and changes in cell type as related to size (that is, attempt to determine whether various cell types convert or are epithelioid cell mutant lines); (4) correlation of the size, cell type, configuration of growth, tendency for blood vessel invasion and extrascleral invasion, and perforation of Bruch's membrane with the clinical appearance and prognosis; (5) specification of the factors resulting in development of inflammation, the type of inflammation, and the relationship of inflammation to clinical course; and (6) determination of changes in retinal

pigment epithelium, Bruch's membrane, retina, and development of neovascularization as related to tumor growth.

Since only limited data are available concerning the natural history of ocular melanoma, particularly small melanomas, studies are also needed for the identification of clinically measurable variables, such as size, location, degree of pigmentation, transillumination, the presence of yellow pigment or drusen, neovascularization, collar button extension, retinal detachment, vitreous or choroidal hemorrhage, and secondary glaucoma; these are important in determining the metastatic potential and prognosis of uveal melanomas. Specifically, there is a need to (1) investigate the potential of melanoma patients to develop metastatic disease when the tumor is left untreated for long periods or when treated by modes other than enucleation, and (2) identify clinical signs that indicate when a small melanoma is entering a rapid growth phase or has an increased potential for metastasis. There is also need to determine the incidence of metastatic disease at the time of the initial diagnosis of choroidal melanoma. The frequency of second neoplasms at other organ sites, including the skin, needs to be established.

Therapy

Beginning in the late nineteenth century, enucleation became the standard and almost universally accepted treatment for all eyes containing a choroidal or ciliary body melanoma (Figure 5). Advocated as early as 1882 by Fuchs, this view was preeminent until recently and continues to have its advocates.[56] However, concern about this mode of therapy is heightened by the differences in conclusions generated by the analysis of Zimmerman and coworkers,[11,12] which appear to indicate that about two-thirds of the fatalities following enucleation could be attributed to the dissemination of tumor emboli at the time of surgery, and the analyses of others[56,57] which appear to argue to the contrary. Thus, there is a need for rigorous tests of the efficacy of any treatments on disease outcome in prospective, randomized controlled clinical trials which would include: plans for standardization of definitions, procedures, and measurement techniques among participating investigators and centers; adequate patient populations; a consideration of prognostic factors and other issues of baseline comparability; and appropriate plans for data collection, monitoring, and analysis.

Patients with extrascleral extension are generally considered to have a poor prognosis. There is some evidence that exenteration of the orbit may yield good results, particularly when performed promptly after recognition that there is residual tumor in the orbit.[58] However, evaluation of the small amount of existing data on this issue is difficult, because it is often not clear from the literature whether cases of apparent extrascleral extension truly have residual tumor in the orbit, or if the tumor only extended close to the line of transsection. There is an urgent need to determine whether adjuvant chemotherapy, radiation therapy, or immunotherapy will improve the prognosis in these cases.

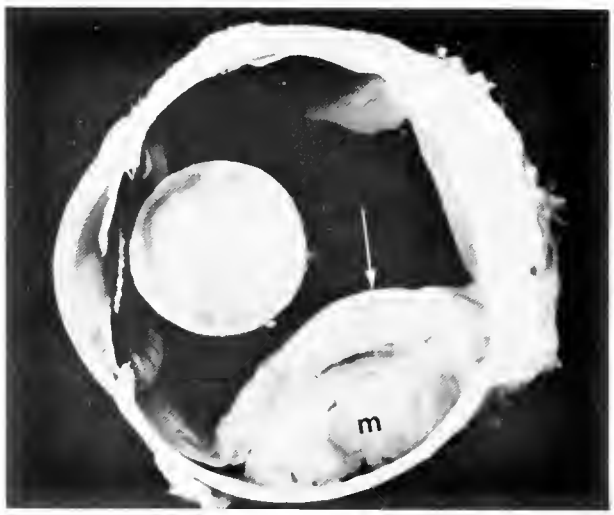


FIGURE 5. An eye removed at surgery which has been cut open revealing a melanoma (m) beneath the retina (arrow).

Cell Biology

Basic research is essential to the development of improved therapy for patients with choroidal melanoma. There is a pressing need for an established human choroidal melanoma cell line for use in biochemical, immunologic, and chemotherapeutic investigations. Ultrastructural studies of human choroidal melanoma should be continued to search for evidence of a viral etiology, develop ultrastructural criteria useful in the cytologic classification, and characterize the local immune response to this tumor.

The development of animal models of choroidal melanoma is of high priority. This objective should be pursued through multiple approaches: (1) heterotransplantation of human choroidal melanoma into the immune deficient nude mouse for use in immunologic and chemotherapeutic studies, (2) induction of choroidal melanoma and other intraocular tumors using oncogenic viruses, and (3) induction of melanoma and other intraocular tumors using chemical carcinogens. Once these models are established, attempts should be made to characterize the natural history, metastatic potential, and immunology of these tumors. Such experimental tumor systems

have considerable value in the evaluation of therapeutic approaches, including different techniques of enucleation and immunotherapy or chemotherapy.

Attempts should be made to characterize tumor-specific immune competence in patients treated by various means at different stages in the natural history of the tumor. Studies of the immunopathology of choroidal melanoma are of considerable importance, that is, ultrastructural and immunologic characterization of immune cells infiltrating the tumor. Investigation into the presence of estrogen receptors and receptors for retinoic acid and related compounds should be undertaken.

Retinoblastoma

It is necessary to improve the ability to distinguish between the sporadic and hereditary retinoblastomas and to be able to predict which family members, especially siblings and offsprings, are at risk of tumor development. Clinical and histopathological evidence suggest that the sporadic type is usually a large single tumor occurring later in the child's development, whereas the hereditary type tends to be small, multifocal, and develop earlier. It would be useful to ascertain through morphologic studies if light microscopic or ultrastructural changes can be correlated with the occurrence of metastases and recurrence of the tumor. In addition, it is important to be able to distinguish second primary tumors from metastases.

Ways of diagnosing retinoblastoma earlier should be sought. This will require a more complete understanding of the immunology of the tumor and the immunologic clues that can be used for diagnosis. It will also require an ability to identify enzyme or chemical changes in the patient's blood and aqueous humor, which would be helpful for diagnosis.

Better methods of treating this tumor are needed. The finding of vitamin A receptors in retinoblastoma and the in vitro demonstration that vitamin A can cause tumor cell death should be explored further with regard to treatment possibilities. In addition, conventional therapy, including chemotherapy and radiotherapy, should be evaluated more systematically, so that statistically meaningful data can be obtained and compared with the results obtained by enucleation (Figure 6). The fact that the mortality of patients with bilateral disease is not significantly different from those with unilateral disease suggests that treatment other than enucleation, as is often employed in the second eye of bilateral retinoblastoma patients, may give equivalent results.

An epidemiologic survey is needed to determine the worldwide incidence of retinoblastoma and the role of genetic, racial, or environmental factors in its etiology. There is the need for further investigation of the cause of retinoblastoma. Knudson's "two hit" theory, the classical autosomal dominant with variable penetrance theory, and even the more recent "three hit" theory must be measured against actual clinical observations to determine which of these is correct.

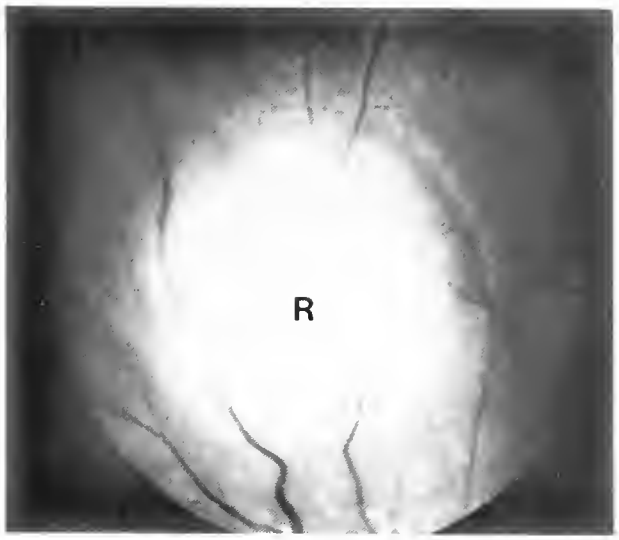


FIGURE 6. The appearance of a small retinoblastoma (R) at a stage in which it can be treated without removing the eye.

The patients with 13q-chromosome need to be more fully examined, for they may hold the key to understanding what role the genetic information in the deleted portion of this chromosome plays in causing tumor formation. With available techniques, it may be possible to isolate this chromosomal segment and determine its biochemical function. This is a unique opportunity that must be pursued.

In addition, further studies are needed to determine the role of viruses in retinoblastoma. Molecular, biophysical, and immunologic studies are needed to look for viral "footprints" and, possibly, the virus itself or viral fragments. Animal models will be useful in determining the role of virus in retinoblastoma.

To increase understanding of the pathogenesis of retinoblastoma, further ultrastructural and biochemical studies should be conducted to identify the cell or cells of origin. Some evidence to date points to the photoreceptor cell, other evidence to the amacrine cell. Both may be precursor cells, or a common precursor retinoblast may exist. Moreover, there is recent evidence that at least some tumors may have a glial origin.[59]

Knowledge of the basis for spontaneous regression of retinoblastoma, which has been estimated to occur in one percent of cases, could be exploited for

clinical purposes. Initial ultrastructural studies suggest that endothelial proliferation, inflammatory cell interaction, and circulating antibodies that may be related to the basophilia in the vessel walls may all be important. Definitive information about the role of each of these factors is needed. In addition, conflicting findings concerning the presence or absence of tumor angiogenesis factor in retinoblastoma have been reported, and this question needs to be resolved.

Better data on tumor patients, that is, accurate case histories and well-preserved tumor material from enucleated eyes are required for the necessary investigations. This requires cooperation among clinicians and between clinicians and basic scientists. Further requirements include: (1) establishment of additional tumor cell lines, (2) identification of spontaneously occurring animal tumors, and (3) further development of induced animal tumors and information about the similarities and differences between these and the human tumors.

The investigations proposed above would require the following types of investigators: ocular oncologists, morphologists with expertise in tumors or in retina, geneticists, molecular biologists or tumor virologists, veterinary ophthalmologists and pathologists, radiobiologists with both clinical and basic research expertise, epidemiologists and biometricians, and biochemists with a knowledge of and interest in the retina.

RECOMMENDATIONS

Based on the foregoing assessment of recent accomplishments, current activities, and research needs and opportunities in "Tumors," the Panel has made the following recommendations concerning research in this subprogram over the next five years. These have been grouped under two headings: Program Base and Program Development Priorities.

The Program Base includes areas of ongoing research where the current level of activity is considered adequate, or areas of ongoing research in which there may be great need for additional activity, but where, in the Panel's judgment, little or no opportunity (new methods or insights) exists at present to justify a significant expansion of effort. Nonetheless, additional applications for research grants in these areas may be funded if they are innovative and of very high quality as determined by the NIH peer review system.

Program Development Priorities include areas of ongoing research in which new knowledge and techniques offer particular opportunities for scientific progress, or promising new areas of research in which there is little or no support at present but where there is both great need and high potential for success. Such areas are judged to warrant significantly increased support over the next five years, provided that high quality applications for research grants in these areas are forthcoming.

Program Base

Ocular Melanoma

- Continue basic and clinical research aimed at better understanding the pathogenesis of ocular tumors and improved methods of diagnosis and treatment.
- Establish continuous cell lines of human choroidal melanoma cells in tissue culture and make available material from human ocular tumors for studying causative factors and the efficacy of certain treatments.

Retinoblastoma

- Conduct morphologic and biochemical studies of retinoblastoma to determine its cellular origin.
- Establish cell lines for basic studies on the pathogenesis and treatment of retinoblastoma.

Program Development Priorities

Ocular Melanoma

- Establish prospective, randomized controlled clinical trials of new treatments for patients with ocular melanomas.
- Characterize the nature of tumor-specific immune competence in treated and untreated patients.
- Develop animal models of ocular melanoma for use in immunologic, biochemical, and therapeutic studies.

Retinoblastoma

- Study genetic factors involved in the development of retinoblastoma.

RESOURCE REQUIREMENTS

After reviewing current research grant support in each of these categories and assessing the need and potential for future development, the Panel has estimated the number of projects it believes are needed to carry out its recommendations in FY 1983. These estimates are shown in the table on the following page. For a discussion of the general basis and significance of these projections, see the "Summary" at the beginning of this report.

RESOURCE TABLE

TUMORS

	No. of Grants FY 1981	Panel Recommendation FY 83	
		Add. Grants	Total Grants
Program Base			
Melanoma			
A. Continue basic and clinical research on the pathogenesis, diagnosis, and treatment of ocular tumors.	11	−6	5
B. Establish continuous cell lines of human choroidal melanoma cells in tissue culture and study human ocular tumors for causative factors and treatment modalities.	0	1	1
Retinoblastoma			
A. Conduct morphologic and biochemical studies of retinoblastoma to determine its cellular origin.	0	1	1
B. Establish cell lines for basic studies on the pathogenesis and treatment of retinoblastoma.	1	1	2
Program Development Priorities			
Melanoma			
A. Establish prospective, randomized, controlled clinical trials of new treatments for patients with ocular melanomas.	0	10*	10
B. Characterize the nature of tumor-specific immune competence in treated and untreated patients.	2	1	3
C. Develop animal models of ocular melanoma for use in immunologic, biochemical, and therapeutic studies.	2	1	3
Retinoblastoma			
A. Study genetic factors involved in the development of retinoblastoma.	2	2	4
Subtotal Grants	**18**	**11**	**29**
(% of Program)	**(5)**	**(10)**	**(6)**
Total Estimated Cost	**$1,887,000**	**$1,158,000**	**$3,045,000**

*Includes one multicenter, randomized controlled clinical trial of selected therapies.

REFERENCES

1. Scotto J, Fraumeni JF, Lee JAH: Melanomas of the eye and other noncutaneous sites. *J Natl Cancer Inst* 56:489–491, 1976.
2. McLean IW, Foster WD, Zimmerman LE: Prognostic factors in small malignant melanomas of the choroid and ciliary body. *Arch Ophthalmol* 95:48–58,1977.
3. Elwood JM, Lee JA, Walter SD, et al: Relationship of melanoma and other skin cancer mortality to latitude and ultraviolet radiation in the United States and Canada. *Int J Epidemiol* 3:325–332, 1972.
4. Robertson DM, Campbell RJ: Errors in the diagnosis of malignant melanoma of the choroid. *Am J Ophthalmol* 87:269–275, 1979.
5. Shields JA: Current approaches to the diagnosis and management of choroidal melanomas. *Surv Ophthalmol* 21:443–463, 1977.
6. Coleman DJ, Abramson DH, Jack RL, et al: Ultrasonic diagnosis of tumors of the choroid. *Am J Ophthalmol* 91:344–354, 1974.
7. Norton EWD, Smith JL, Curtin VT, et al: Fluorescein fundus photography: An aid in the differential diagnosis of posterior ocular lesions. *Trans Am Acad Ophthalmol Otolaryngol* 68:755–765, 1964.
8. Michelson JB, Felberg NT, Shields JA: Carcinoembryonic antigen: Its role in the evaluation of intraocular malignant tumor. *Arch Ophthalmol* 94:414–416, 1976.
9. Char DH: Inhibition of leukocyte migration with melanoma-associated antigen in choroidal tumors. *Invest Ophthalmol Vis Sci* 16:176–179, 1977.
10. Brownstein S, Phillips TM, Lewis MC: Specificity of tumor-associated antibodies in serum of patients with uveal melanoma. *Can J Ophthalmol* 13:190–193, 1978.
11. Zimmerman LE, McLean IW: An evaluation of enucleation in the management of uveal melanomas. *Am J Ophthalmol* 87:741–760, 1979.
12. Zimmerman LE, McLean IW, Foster WD: Does enucleation of the eye containing a malignant melanoma prevent or accelerate the dissemination of tumor cells? *Br J Ophthalmol* 62:420–425, 1978.
13. Davidorf FH, Makley TA, Lang JR: Radiotherapy of malignant melanoma of the choroid. *Trans Am Acad Ophthalmol Otoloryngol* 81:849–861, 1976.
14. Gragoudas ES, Goitein M, Koehler AM, et al: Proton irradiation of small choroidal malignant melanomas. *Am J Ophthalmol* 83:665–673, 1977.
15. Vogel MH: Treatment of malignant choroidal melanomas with photocoagulation: Evaluation of one-year follow-up data. *Am J Ophthalmol* 74:1–11, 1972.
16. Lincoff H, McLean J, Long R: The cryosurgical treatment of intraocular tumors. *Am J Ophthalmol* 63:389–390, 1977.
17. Albert DM, Sang DN: Retinoblastoma and pseudoglioma, in Frayer W (ed): *Lancaster Course in Ophthalmic Histopathology*. Philadelphia, FA Davis, Inc, 1980.
18. Tarkinnen A, Tuovinen E: Retinoblastoma in Finland 1912–64. *Acta Ophthalmol (Copenh)* 49:293, 1971.
19. Albert DM, Lahav M, Lesser RL, et al: Recent observations regarding retinoblastoma: I. Ultrastructure, tissue culture growth, incidence and animal models. *Trans Ophthalmol Soc UK* 94:909–928, 1974.
20. Kodilinye HC: Retinoblastoma in Nigeria: Problems of treatment. *Am J Ophthalmol* 63:469–481, 1967.
21. Bras G, Cole H, Ashmeade-Dyer A, et al: Report on 151 childhood malignancies observed in Jamaica. *J Natl Cancer Inst* 43:417–421, 1969.
22. Jensen RD, Miller RW: Retinoblastoma: Epidemiologic characteristics. *N Engl J Med* 285:307–311, 1971.
23. Maklin RD: A study of retinoblastoma in Ohio. *Am J Hum Genet* 12:1–43, 1960.
24. Fraser CR, Friedman AI: *The Causes of Blindness in Children*. Baltimore, Johns Hopkins Press, 1967.
25. Kupfer C: Retinoblastoma treated with intravenous nitrogen mustard. *Am J Ophthalmol* 36:1721, 1953.
26. Char DH, Castro JR: Helium ion therapy for choroidal melanoma. *Arch Ophthalmol* 100:935–938, 1982.
27. Albert DM, Puliafito CA, Fulton AB, et al: Increased incidence of choroidal malignant melanoma occurring in a single population of chemical workers. *Am J Ophthalmol* 89:323, 1980.
28. Albert DM, Wagoner MD, Moazed K, et al: Heterotransplantation of human choroidal melanoma into the athymic "nude" mouse. *Invest Ophthalmol Vis Sci* 19:555–559, 1980.
29. Albert DM, et al: Induction of ocular neoplasms in Fischer rats by intraocular injection of nickel subsulfide. *Invest Ophthalmol Vis Sci*, to be published.
30. Albert DM, et al: Feline uveal melanoma model induced with feline sarcoma virus. *Invest Ophthalmol Vis Sci*, to be published.
31. Tso MOM, Zimmerman LE, Fine BS: The nature of retinoblastoma: I. Photoreceptor differentiation: A clinical and histopathologic study. *Am J Ophthalmol* 69:339–349, 1970.
32. Popoff M, Ellsworth RM: The fine structure of retinoblastoma: In vivo and in vitro observations. *Lab Invest* 25:389–402, 1971.
33. Albert DM, Dalton AJ, Rabson AS: Microtubules in retinoblastoma. *Am J Ophthalmol* 69:296–299, 1970.
34. Mullaney J: DNA in retinoblastoma. *Lancet* 2:918, 1968.
35. Knudson AG Jr: Mutation in cancer: Statistical study of retinoblastoma. *Proc Natl Acad Sci USA* 68:820–823, 1971.
36. Abramson DH, Ellsworth RM, Zimmerman LE: Nonocular cancer and retinoblastoma survivors. *Trans Am Acad Ophthalmol Otolaryngol* 81:454–457, 1976.
37. Little JB, Weichselbaum RR, Nove J, et al: X-ray sensitivity of fibroblasts from patients with retinoblastoma and with abnormalities of chromosome 13, in Friedberg LF, Hanawalt PC (eds): *DNA Repair Mechanisms*. New York, Academic Press, 1978.

38. Shields JA, Leonard BC, Michelson JB, et al: B-scan ultrasonography in the diagnosis of atypical retinoblastoma. *Can J Ophthalmol* 11:42–51, 1976.
39. Abramson D, Piro P, Ellsworth R, et al: Lactic acid dehydrogenase and isoenzyme patterns. *Arch Ophthalmol* 97:870, 1979.
40. Dias P: Prognostic significance of aqueous humor lactic acid dehydrogenase activity. *Br J Ophthalmol* 63:571, 1979.
41. Reid TW, Albert DM, Rabson AS, et al: Characteristics of an established cell line of retinoblastoma. *J Natl Cancer Inst* 53:347–360, 1974.
42. McFall RC, Sery FW, Makadon M: Characteristics of a new continuous cell line derived from human retinoblastoma. *Cancer Res* 37:1003–1007, 1977.
43. Wiggert B, Russell P, Lewis M, et al: Differential binding to soluble nuclear receptors and effects on cell viability of retinol and retinoic acid in cultured retinoblastoma cells. *Biochem Biophys Res Commun* 79:218–225, 1977.
44. Russell P, Wiggert B, Chager G: Separation of retinoic receptors from cultured retinoblastoma cells. *Biochim Biophys Acta* 543:586–589, 1978.
45. Russell R, Wiggert B, Derr J, et al: Nuclear uptake of retinoids: Autoradiographic evidence in retinoblastoma cells in vitro. *J Neurochem* 34:1557–1560, 1980.
46. Saari JC, Heffernan T, Fulterman S, et al: Retinoid binding proteins in retinoblastoma cells. *Invest Ophthalmol Vis Sci* 17(suppl):254, 1978.
47. Saari JC, Futterman S, Stubbs GW, et al: Cellular retinol and retinoic acid-binding proteins in transformed mammalian cells. *Invest Ophthalmol Vis Sci* 17:988–992, 1978.
48. Wiggert B, Bergsma D, Lewis M, et al: Vitamin A receptors: Retinol binding in neural retina and pigment epithelium. *J Neurochem* 29:947–954, 1977.
49. Sporn M, Dunlop N, Newton D, et al: Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids). *Fed Proc* 35:1332–1338, 1976.
50. Mukai N, Nakajima T, Freddo T, et al: Retinoblastoma-like neoplasm induced in C3H/BifB/Ki strain of mice by human adenovirus serotype 12. *Acta Neuropathol (Berl)* 39:147–155, 1977.
51. Albert DM, Lahav M, Colby ED, et al: Retinal neoplasia and dysplasia. Induction by feline leukemia virus. *Invest Ophthalmol Vis Sci* 16:325–337, 1977.
52. Graham BJ, Duane TD: Ocular melanoma task force report. *Am J Ophthalmol* 90:728–733, 1980.
53. Jensen OA: Malignant melanoma of the human uvea: Recent follow-up of cases in Denmark, 1943–1952. *Acta Ophthalmol (Copenh)* 48:1113–1128, 1970.
54. Jensen OA: Malignant melanoma of the uvea in Denmark, 1943–1952: A clinical, histopathologic and prognostic study. *Acta Ophthalmol (Copenh)* 75(suppl):1–220, 1963.
55. Gass JDM: Problems in the differential diagnosis of choroidal nevi and malignant melanomas. *Am J Ophthalmol* 83:299–323, 1977.
56. Manschot WA, von Peperzeel HA: Choroidal melanoma: Enucleation or observation? A new approach. *Arch Ophthalmol* 98:71–77, 1980.
57. Seigel D, Meyers M, Ferris F III, et al: Survival rates after enucleation of eyes with malignant melanoma. *Am J Ophthalmol* 87:761–765, 1979.
58. Shammas HF, Blodi FC: Orbital exenteration of choroidal and ciliary body melanomas. *Arch Ophthalmol* 95:2002–2005, 1977.
59. Tso MOM: Clues to the cells of origin in retinoblastoma. *Int Ophthalmol Clin* 20:191–210, 1980.

DEGENERATIVE DISORDERS OF THE RETINA

4

DEVELOPMENTAL AND HEREDITARY DISORDERS

INTRODUCTION

DEVELOPMENTAL AND HEREDITARY disorders of the retina and choroid afflict newborn and young children and instill fear in many prospective parents, especially those with known family histories of these diseases. For families with affected offspring, there is the immediate emotional impact of learning that they have an affected child and the long-range effect on these families and society as they try to help blind or partially sighted children grow up in the world of the sighted.

Young adults are equally disheartened when they discover that they have a degenerative retinal disorder, which was probably present from birth and will eventually lead to a total loss of vision. In retinitis pigmentosa, one of the more common hereditary retinal degenerations, a ring-like area of blindness slowly encroaches on the central island of vision. Patients with this disease often carry the burden of uncertainty as to how long they will retain their remaining central vision. Some of these patients have excellent central vision but are severely handicapped by loss of side vision and resulting impaired mobility. An estimated 3 to 5 percent of patients with retinitis pigmentosa are born profoundly deaf and usually discover in young adulthood that they also face inevitable loss of vision.

Some developmental and hereditary disorders of the retina and choroid have been named on the basis of their appearance as viewed with an ophthalmoscope; for example, in retinitis pigmentosa, irregular deposits of pigment can be seen around the midperipheral retina as the retina deteriorates. In addition, the diseases are classified, according to the mode of inheritance, into autosomal dominant, autosomal recessive, and X-chromosome-linked types. Some conditions with minimal, if any, changes visible with the ophthalmoscope are named on the basis of the degree and type of visual impairment. Thus, rod-mediated function is profoundly affected and cone-mediated function is relatively intact in many stationary (nonprogressive) forms of night blindness, whereas loss of cone function with intact rod function exists in congenital achromatopsia. Degenerative changes can selectively affect patches of peripheral retina, as in sector retinitis pigmentosa, or they can lead to loss of cone and rod function in the central retina as in juvenile hereditary macular degeneration.

These diseases can be considered also in terms of whether they appear to involve the outer layers of the retina and/or choroid, as occurs in retinitis pigmentosa, gyrate atrophy of the choroid and retina, choroideremia, and congenital amaurosis of Leber; or involve the inner layers of the retina, as occurs in some forms of stationary night blindness and in juvenile sex-linked retinoschisis. In a developmental disorder, called retinal dysplasia, affected infants have maldevelopment of the retina; retinal dysplasia can be accompanied by maldevelopment of the eye and chromosomal aberrations. Some diseases are named after the physicians who first recognized the association of a retinal degeneration with other findings, as, for example, Usher's syndrome, in which Usher noted the association of retinitis pigmentosa with profound deafness and a disorder of balance. More information is needed on the cellular localization of the primary defects as well as the underlying biochemical abnormalities before these diseases can be reclassified in terms of etiology.

The National Society to Prevent Blindness estimates that some 1,450 new cases of legal blindness due to retinitis pigmentosa were reported in the

United States in 1978.[1] An estimated 50,000 to 100,000 people are afflicted in this country alone.[2] Approximately 1 in 50 people in this country carry the gene(s) for these disorders even though they enjoy normal vision.[3] If two carriers with the same gene for autosomal recessive retinitis pigmentosa marry, they have a 1 in 4 chance with each childbirth of having an affected child. All social, ethnic, and racial groups are affected, and more than 50 percent of the patients with these conditions report a negative family history. The cost of these disorders to society is staggering, particularly in view of the fact that no effective therapy is available for practically all types and that some patients may become legally blind by age 40 or even earlier.[1]

The state of knowledge in this field has increased substantially in the past 5 to 10 years. First, much more is known about the ultrastructure, function, and biochemistry of the normal retina and pigment epithelium, and this knowledge has been applied with remarkable success to an understanding of disease processes. Second, patients with heredodegenerative retinal diseases are being studied with sophisticated noninvasive techniques, which provide new insights into the sites of abnormal function. Third, educational programs have resulted in a marked increase in the availability of postmortem donor eyes, and biochemical studies of blood, urine, and cultured skin fibroblasts have led to new information on the causes of some of these conditions and in some instances to treatment trials. Fourth, in animals with hereditary retinal diseases, delineation of specific biochemical defects in the photoreceptors or defects in the relationships of one cell to another have provided a basis for suggesting similar pathogenetic mechanisms in human disease.

Whether the disease process in any of the available animal models has the same cytogenetic basis as that encountered in the human disease is as yet unknown. One difficulty in establishing this relationship is the fact that specific biochemical defects have not been identified in the great majority of patients with these diseases. Moreover, because of the close functional relationship between adjacent cells in the retina, it is frequently difficult to identify the cell in which the mutant gene is expressed.

The genetic factors also have been difficult to unravel. The precise loci on the chromosomes that lead to retinal diseases have not been established, so that the chromosomal segments that are abnormal are not yet available for study. Prenatal tests or methods to detect carriers of these conditions are not available for most of these diseases; therefore, genetic counseling can be given to families only after they have had an affected child. Although the long-term course of progressive forms of hereditary disorders of the retina and choroid are known (that is, the eventual visual outcome can be predicted to within a decade), the short-term natural histories of these conditions over 1 to 5 years remain to be documented. However, some of these conditions may appear to be relatively stable for 5 to 10 years on routine ocular examination. More information on short-term natural histories is needed to determine whether future trials of treatment aimed at stabilizing these conditions can be evaluated over the short term.

Research on developmental and hereditary disorders of the retina and choroid is closely allied to research within other subprograms of the NEI Retinal and Choroidal Diseases program. Studies of the cell biology of photoreceptors and retinal pigment epithelium, the interaction of cells within the retina, and mechanisms of visual adaptation are particularly relevant.

Inherited degenerations of the retina and choroid are often associated with myopia or astigmatism, and therefore, research on these diseases may be relevant to the broader questions of what factors control the expression of myopia and astigmatism.

The effects of drugs and other agents on the retina and choroid, which may produce degeneration or modify function, are directly related to this research area. Research on the effects of nutrition on the eye is also relevant, as it is well known that specific nutritional deficiencies can lead to retinal degenerations. Degenerative diseases may possibly represent premature aging of the retina, and therefore, they may serve as models of the biology of aging in the retina as well as the brain. Because the retina is developmentally an extension of the brain, research on retinal degenerations may prove relevant to understanding diseases that affect other parts of the nervous system.

SUBPROGRAM OBJECTIVES

- To discover the specific causes of retinal degenerations in humans and in appropriate animal models.
- To develop the techniques needed to identify affected patients and seek techniques for prenatal diagnosis.
- To define the natural histories of these diseases.
- To conduct therapeutic trials where appropriate.

OVERVIEW OF CURRENT RESEARCH SUPPORT

The National Eye Institute supported 42 grants in FY 1981, at a total cost of $3.8 million, for research on Developmental and Hereditary Disorders of the retina and choroid. This support has permitted electrophysiological and psychophysical testing and fundus reflectometry measurements on patients with retinal degenerations and correlation of results of these tests with ultrastructural and biochemical studies on postmortem donor eyes from patients with these diseases. Grant support has also been used to study normal and postmortem human donor retinas and/or pigment epithelium using ultrastructural, biochemical, and tissue culture techniques.

Basic research on the aging mammalian retina and pigment epithelium and on the developing visual system also has been in progress. Animal models of hereditary retinal degeneration including those in mice,[4,5] Royal College of Surgeons (RCS)[6] and Wag-Rij[7] rats, Irish setters,[8] English setters,[9,10] miniature French poodles,[11] Alaskan malamutes,[12] chickens,[13] collies,[14] baboons,[15] and monkeys[16] are under study. Research support also has been directed toward the biochemical delineation of defects in human hereditary retinal diseases such as gyrate atrophy of the choroid and retina. A treatment trial for patients with gyrate atrophy with vitamin B[6] and/or a low-protein, low-arginine diet has been initiated in several research centers. Research is continuing also on the pathogenesis of neuronal ceroid lipofuscinosis (Batten-Mayou disease) and other hereditary diseases with both systemic and ocular manifestations such as the sphingolipidoses, the mucopolysaccharidoses, and Refsum's disease.

Support by the National Eye Institute has remained the major resource for these endeavors, but additional support also has been provided by the Veterans Administration and private foundations, notably Fight for Sight, Inc., National Retinitis Pigmentosa Foundation, National Society to Prevent Blindness, Inc., Research to Prevent Blindness, Inc., and March of Dimes.

RECENT ACCOMPLISHMENTS

Early Diagnosis

The electroretinogram (ERG) has provided physiological criteria for establishing the diagnosis of retinitis pigmentosa in early life, even at a time when fundus abnormalities visible with the ophthalmoscope are minimal or absent.[2,17–20] Patients with widespread progressive forms of retinitis pigmentosa have shown not only reduced amplitudes, but also delays in the cone and/or rod b-wave implicit times (that is, the time interval between stimulus onset and the major cornea positive peak of the cone or rod response); in contrast, patients with self-limited sector retinitis pigmentosa or stationary forms of night blindness have had reduced ERG amplitudes with normal b-wave implicit times.[2] In families with retinitis pigmentosa, ERGs have been used to identify which members are affected and which are normal. Family members age 6 and over, with normal cone and rod amplitudes and normal cone and rod b-wave implicit times, have not later developed retinitis pigmentosa.

Early diagnosis has been helpful in establishing hereditary patterns in some families and identifying families at high risk for having more affected children. Moreover, early diagnosis has proved to be important in at least one hereditary disease associated with retinitis pigmentosa, hereditary a-beta-lipoproteinemia. A patient with this rare disease detected early in life had his ERG changed to normal with large doses of vitamin A, while a patient with a more advanced stage of this disease did not respond.[21] Knowledge of long-term visual prognoses also has been useful for patients and families seeking vocational counseling.

Detection of Carriers and Prenatal Diagnoses

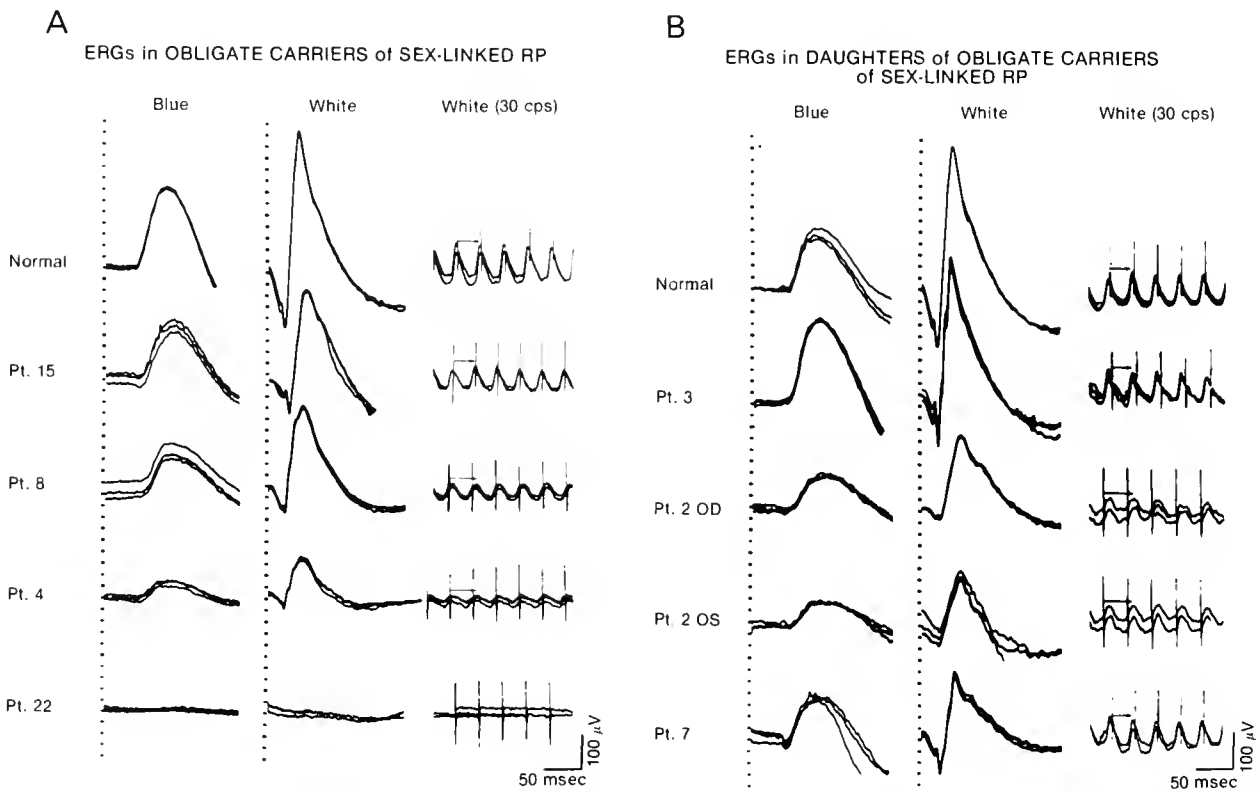
About one-third of the outpatient population with retinitis pigmentosa are males, who have no affected female relatives. It is important to know whether they have autosomal recessive or X-chromosome-linked disease, for patients with autosomal recessive disease characteristically retain vision until age 45 to 60, while those with X-chromosome-linked disease usually are virtually blind by age 30 to 40. If the mother or daughter of an affected male shows a patch of bone spicule pigment in the periphery or a tapetal-like reflex in the macula, she can be considered an obligate carrier, and the affected males in her family can be typed as X-chromosome-linked. However, many obligate carriers of X-chromosome-linked disease, identified through pedigree studies, have shown no visible fundus abnormalities.

Within the past five years, studies of females in families with known X-chromosome-linked disease have shown that the obligate carriers could almost always be detected on the basis of an abnormal full-field ERG. In 96 percent of the obligate carriers tested, the ERG amplitudes were reduced with normal or delayed cone ERG implicit times in one or both eyes (Figure 1A). Only one-half of these patients had an abnormal fundus appearance. Daughters of obligate carriers had either normal ERGs, or abnormal ERGs similar to those of

obligate carriers (Figure 1B). In contrast, carriers of autosomal recessive disease had a normal fundus appearance and normal full-field ERGs.[22] In addition to the ERG studies, some carriers have been detected with early receptor potential testing,[23] fundus reflectometry,[24] or vitreous fluorophotometry.[25]

Once the mode of transmission has been established as X-chromosome-linked by detection of carrier females, ERG screening can be extended to their sisters and offspring, and genetic counseling can be provided. Females identified as carriers of X-chromosome-linked retinitis pigmentosa could be told that they have a 50 percent chance with each male childbirth of having an affected son and a 50 percent chance with each female childbirth of having a carrier daughter. Their affected male relatives would know that they have a poor long-term visual prognosis, and that all their sons would be normal and all their daughters would be carriers. Detection and effective genetic counseling of both carrier females and affected males in families with X-chromosome-linked disease could potentially reduce the incidence of retinitis pigmentosa in the United States by 6 to 10 percent.[26]

Another carrier state that can be detected on the basis of recent research is the carrier of gyrate atrophy of the choroid and retina. Carriers of this autosomal recessive disorder have a partial reduc-



FIGURES 1A and B. ERG responses from a normal subject and four obligate female carriers of X-chromosome-linked (sex-linked) retinitis pigmentosa (A) and from a normal subject and three daughters of obligate carriers of X-chromosome-linked retinitis pigmentosa (B). Pt (patient) 15, age 39; Pt 8, age 41; Pt 4, age 51; Pt 20, age 70; Pt 3, age 23; Pt 2, age 21; Pt 7, age 20. Pt 2 had bone spicule pigmentation only in OS. Pt 3 and Pt 7 had normal fundus examinations. For both A and B, rod isolated responses to blue light are illustrated in column 1, combined cone and rod responses to white light under dark-adapted conditions are illustrated in column 2, and cone isolated responses to 30 cycles per second (cps) white flickering light are illustrated in column 3 for the normals and for those patients with detectable responses. Stimulus onset is designated by the vertical hatched lines in columns 1 and 2; the vertical lines and the arrows in column 3 designate cone b-wave implicit times. Obligate female carriers (A) showed reduced amplitudes to blue light (i.e., $< 125\ \mu V$) and/or white light (i.e., $< 350\ \mu V$) under dark-adapted conditions with or without delays in cone b-wave implicit times (normal range 2532 msec) to 30 cps white flicker in one or both eyes. Daughters of obligate carriers had either normal responses (for example, Pt 3) or abnormal responses (for example, Pt 2 and Pt 7) similar to those of obligate carriers. (From Berson EL, Rosen JB, and Simonoff EA. *Am J Ophthalmol* 87:460, 1979. Published with permission from the American Journal of Ophthalmology. Copyright by the Ophthalmic Publishing Company.)

tion of ornithine ketoacid-transaminase in cultured skin fibroblasts or lymphocytes.[27-29] Prenatal diagnosis also may be possible, for this enzyme is normally present in cultured cells from the amniotic fluid.

Biochemical abnormalities have been defined in many inherited metabolic diseases associated with abnormal storage of lipid and grouped under the general heading of familial sphingolipidoses; diseases that involve the eye as well as other organs include Tay-Sachs disease, generalized GM[1] gangliosidosis, Niemann-Pick disease, and Fabry's disease. A cherry-red spot in the macula due to infiltration of lipid into parafoveal ganglion cells is the most striking fundus lesion often seen in the first three conditions, whereas tortuosity of retinal vessels has been reported in the fourth. The defective enzymes in these diseases are, respectively, hexoseaminidase A, beta-galactosidase, sphingomyelinase, and ceramide trihexoxide-alpha-galactosidase. All four of these fatal hereditary conditions can now be detected prenatally.[30]

Therapeutic Trials

Patients with gyrate atrophy of the choroid and retina have myopia, constricted visual fields, elevated dark-adaptation thresholds, small or nondetectable electroretinograms and chorioretinal atrophy distributed around the peripheral fundus and sometimes near the optic disc (Figure 2). Initially, the patients can be asymptomatic; the diagnosis may be made at the time of a routine check for eyeglasses. Patients develop cataracts and usually become virtually blind between ages 40 to 55 due to extensive chorioretinal atrophy. Although this condition was first described in 1896,[31] little was known until recently about the underlying biochemical abnormalities or possible avenues for therapy.

The rapid recent progress made in understanding gyrate atrophy has been based on a logical sequence of investigation starting first with the detection of elevated ornithine in the blood of patients with this disease[32] and then proceeding to delineation of an enzyme defect, namely a deficiency of ornithine ketoacid-transaminase, in cultured skin fibroblasts or lymphocytes.[27-29,33-35] Vitamin B^6, a cofactor for ornithine ketoacid-transaminase, was shown to increase the activity of this enzyme in cultured fibroblasts from a patient,[29] and in vivo administration of this vitamin lowered plasma ornithine by 50 percent.[34,36] However, heterogeneity was found to exist, for some patients did not respond to vitamin B^6.[34,36,37]

Knowledge that arginine is a precursor of ornithine has led to the idea that a low arginine diet could effectively lower plasma ornithine levels.[38] Laboratory animal studies showed that high doses of ornithine injected into the vitreous humor are toxic to the pigment epithelium,[39] and supported the hypothesis that if ornithine levels could be lowered substantially, the retinal degeneration associated with gyrate atrophy would also be modified.

All patients so far studied have shown a decline in plasma ornithine levels of 50 percent or more when placed on a low protein (15 mg. per day), low arginine diet or vitamin B^6.[34,36-38,40-43] One patient on this diet, who could tolerate the extreme protein restriction and whose plasma ornithine level was lowered to near-normal levels,[41,42] showed an im-

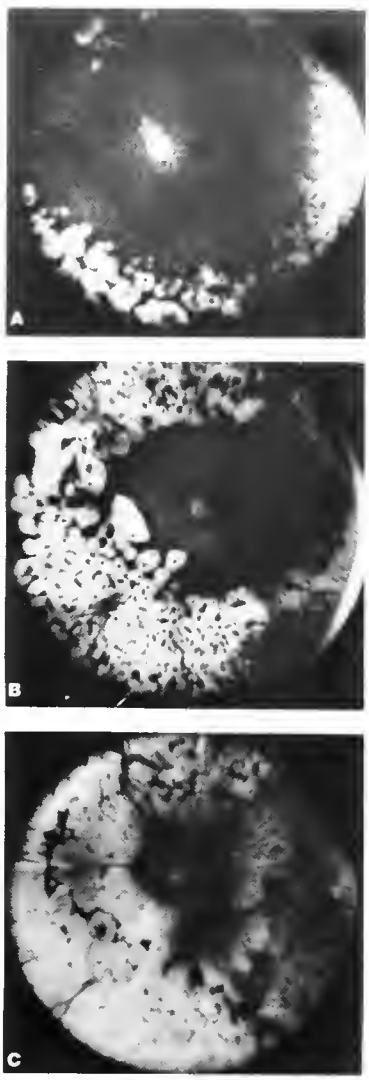


FIGURE 2. Wide-angle fundus photographs of three patients identified as A, B, and C with gyrate atrophy of the choroid and retina. Pt. A, age 14, OS, VA = 20/25; Pt. B, age 10, OS, VA = 20/30; Pt. C, age 20, OS, VA = 20/100. Patient A has the pyridoxine (vitamin B_6) responsive variant of gyrate atrophy and Pts. B and C the pyridoxine nonresponsive variant. (From Berson EL, Schmidt SY, and Shih VE. *Ophthalmology* 85:1018, 1978).

provement in amplitude of the electroretinogram, dark-adaptation thresholds, and color vision after one and one-half years. Another case with improvement in visual acuity has been reported.[43] However, visual function in the majority of patients has not improved despite lowered plasma ornithine, and it still remains to be established whether any degree of biochemical responsiveness to this diet, vitamin B^6, or both, alters the long-term course of this condition. Some patients in these therapeutic trials have remained stable after one year,[40] but considering the natural history of the ocular disease[34,44] about two years will be required to show whether stabilization has occurred in young patients.

The retinal degeneration observed in Refsum's disease[45] also may be amenable to treatment.[46] Patients with this autosomal recessively inherited disorder characteristically have ataxia, elevated serum phytanic acid (a long-chain fatty acid),[47] and elevated cerebrospinal fluid protein with a normal cell count. Ocular findings include an atypical retinitis pigmentosa-like appearance of the peripheral fundus and reduced or nondetectable electroretinograms.

Some patients may have loss of the sense of smell, deafness, pupillary abnormalities, lens opacities, electrocardiographic malfunction, skeletal abnormalities, and unusual dryness of the skin. Diagnosis depends ultimately on the demonstration of an elevated level of phytanic acid in the serum. Affected patients have a defect in alpha-hydroxylation of phytanic acid, and this enzyme deficiency can be detected in extracts of cultured skin fibroblasts. Pathogenesis of this disease may involve replacement of long chain fatty acids, phospholipids, and triglycerides with phytanic acid derived from dietary sources. Abnormal accumulation of phytanic acid in many tissues then leads to malfunction. Treatment with a low phytol, low phytanic acid diet, that is, excluding green leafy vegetables, animal fats, and milk products, has led to lower serum phytanic acid levels, improved nerve conduction times, and lower cerebrospinal fluid protein levels.[46,48–50] Studies are in progress to learn whether dietary treatment will result in improved or stabilized retinal function over the long term.

The possibility of replacing missing enzymes has been considered for patients with some of the familial sphingolipidoses. Enzymes isolated from human placental tissue have been injected intravenously into affected patients. This causes a decrease in the amount of ceramide trihexoxide in the blood of patients with Fabry's disease and the removal of accumulated lipid from the liver and circulation of patients with Gaucher's disease.[30,51]

However, when a trial of hexoseaminidase A was attempted in Tay-Sachs disease, no evidence was obtained that the injected enzyme crossed the blood-brain barrier, and no clinical improvement was noted.[52] More recent investigations have shown that the blood-brain barrier can be opened and that under these conditions the enzyme injected intravenously can enter the brain.[53] However, more studies are needed before these procedures can be advocated for clinical trials on Tay-Sachs disease and other disorders that cause brain and retinal damage.[30]

Clinicopathologic Correlations

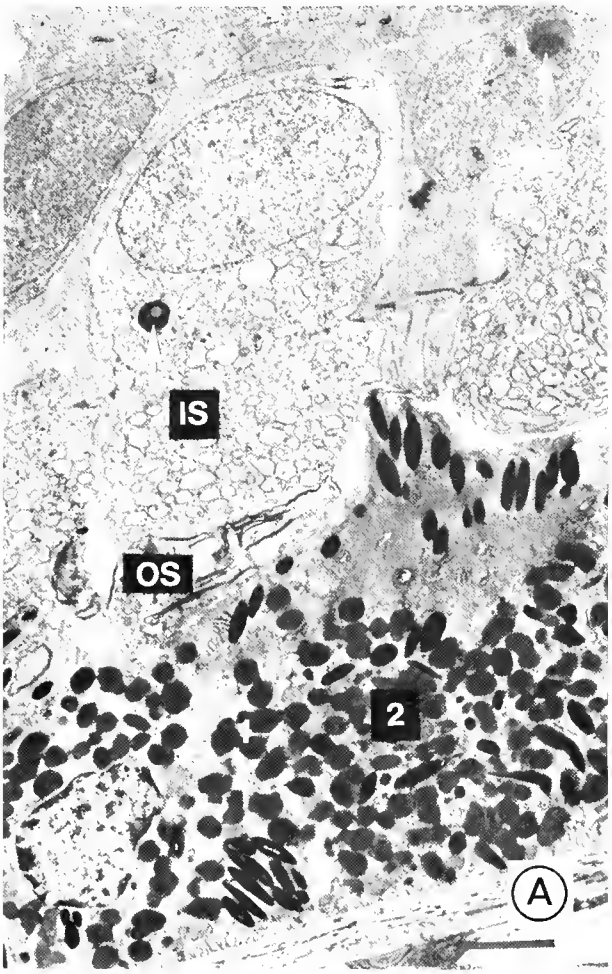
Over the past five years, eyes from patients with retinitis pigmentosa have been obtained shortly after death, and this has allowed study of ultrastructural abnormalities with the electron microscope.[54–56] Remaining cone photoreceptors (Figures 3A and B) had shortened outer segments[54–56] or no organized outer segments and contained autophagic vacuoles in the perinuclear cytoplasm.[55,56] Large numbers of melanolysosomes were observed in the pigment epithelium,[55,56] and a preretinal membrane emanated from the optic disc.[57]

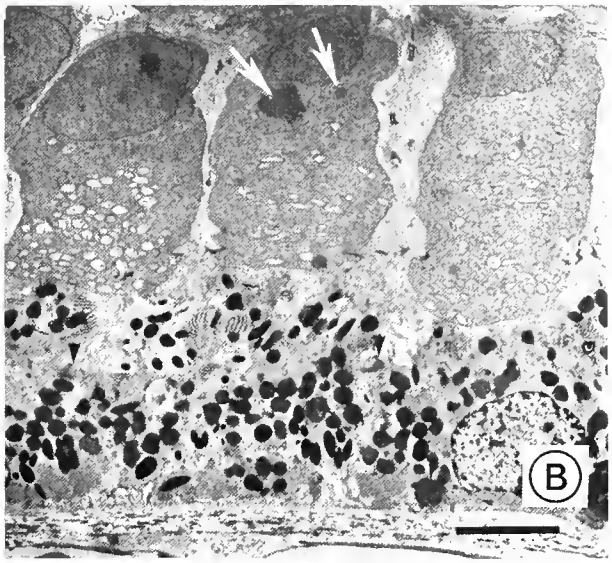
In a donor eye from a 24-year-old male with X-chromosome-linked retinitis pigmentosa, shortened rod outer segments were found in the periphery at a time when these rods were otherwise intact (Figure 3C). The histologic findings could be correlated with premortem clinical findings in this patient based on noninvasive tests of retinal function, supporting the suggestion that these tests provide a reliable index of the extent and type of photoreceptor involvement in this disease.[56]

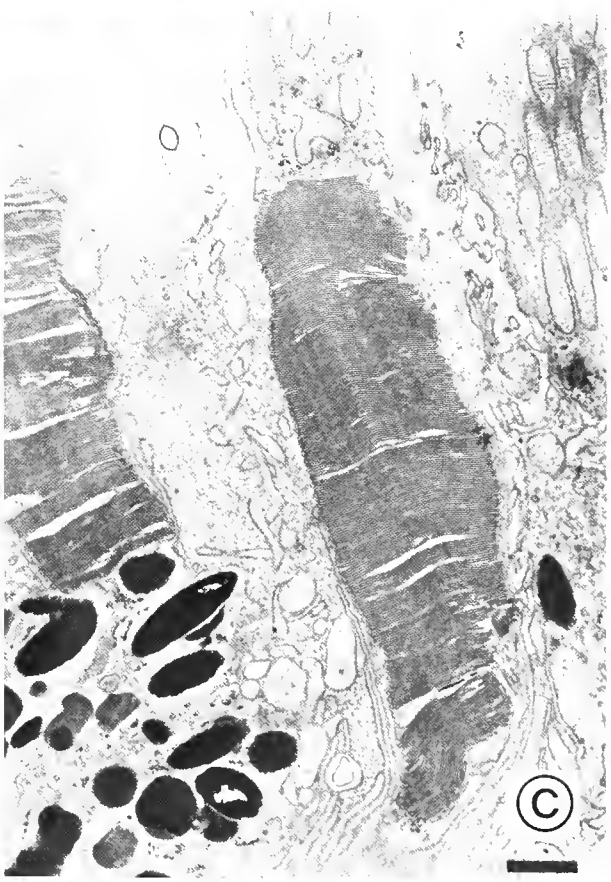
Of interest has been the observation that patients with retinitis pigmentosa could retain considerable visual function even when only cone photoreceptor cells without organized outer segments were visible over large areas. The histologic findings in these donor eyes, combined with measurements of the early receptor potential, fundus reflectometry measurements, and dark adaptation thresholds in young patients with these diseases, suggest an imbalance between renewal of photoreceptor outer segments and outer segment degradation by the pigment epithelium, although the etiology for this imbalance is not clear.[58]

Studies of normal postmortem human donor eyes have recently been expanded to obtain baseline information on the normal retina and pigment epithelium which could be used for comparison with tissue from postmortem donor eyes of patients with retinal degenerations.[59] These studies have shown that photoreceptor-specific processes, such as synthesis of rhodopsin and the high affinity uptake mechanism for taurine, may continue for at least four hours after death.

The ability to culture human pigment epithelium for biochemical and ultrastructural studies has provided a new dimension to efforts aimed at understanding these diseases. Pigment epithelial cells from human eyes have been cultured[60–62] 40 hours after the donor's death[61,62] and cell lines established for







FIGURES 3A, B, and C. Representative sections from a postmortem donor eye from a 24-year-old patient with X-chromosome-linked retinitis pigmentosa: (A) cones in the central fovea have enlarged inner segments (IS) and distorted remnants of outer segments (OS). Autophagic vacuoles (arrows) are seen in the perinuclear cytoplasm. Pigment epithelial cells contain large numbers of melanolysosomes (1), lysosomes (2), and few free melanin granules. Apical protrusions of these cells extend between cone inner segments. Horizontal bar (lower right) is 2 μm. (B) Parafoveal cones have no organized outer segments, and small portions of their inner segments extend beyond the external limiting membrane (X). Autophagic vacuoles (arrows) are present in cone cell bodies. Protrusions of the pigment epithelial cells extend proximal to the apical tight junctions (arrowheads). Horizontal bar (lower right) represents 5 μm. (C) Representative rods in the far periphery have outer segments shortened in length with well-ordered discs. Outer segments are surrounded by microvillous processes of the pigment epithelium. Some processes extend up to the inner segments and are distended. Free melanin granules are prominent in the apical portion of the pigment epithelium. Horizontal bar (lower right) is 1 μm. (From Szamier RB, Berson EL, Klein R, and Meyers S. *Invest Ophthalmol Vis Sci* 18:145, 1979).

biochemical studies.[62,63] Best results have been obtained with eyes removed within five hours after death and placed on ice.[62] In vitro growth characteristics and morphology of the human retinal pigment epithelium have been characterized,[64,65] as well as the increase in the content of lipofuscin with age.[66,67] Cultured pigment epithelial cells from a donor eye with X-linked retinitis pigmentosa synthesize and secrete glycosaminoglycans in a pattern comparable with that of cultured cells from normal eyes.[62] Regional differences (that is, comparing the macula with midperiphery and far periphery) in melanin and lipofuscin content of the pigment epithelium[65,68] and cyclic nucleotide levels[69] have been observed in normal pigment epithelium and they provide baseline information for comparison with cultured pigment epithelium from donor eyes with retinitis pigmentosa.

Animal Models of Hereditary Retinal Disease

Considerable progress has been made in understanding the pathogenesis of retinal degenerations in several animal models. Animals with inherited blindness are a valuable resource in the quest for biochemical abnormalities that cause visual cell degeneration. They are particularly useful in studying the processes of cell differentiation and cell-to-cell interactions. In both human and animal disorders, the process by which immature cells are transformed into specialized photoreceptors (differentiation) may be abnormal, and in some cases, the coordinated interaction between visual cells and pigment epithelium may be faulty. An experimentally derived catalogue of inherited defects is needed for the animal disorders; it could provide a basis for understanding human disease and initiating treatment trials.

Studies of mice with hereditary retinal degeneration (rd mice) have shown that an abnormality in the metabolism of cyclic nucleotides occurs before the visual cells begin to degenerate.[70] These mice have a deficiency in cyclic GMP phosphodiesterase activity which results in the accumulation of cyclic GMP within affected photoreceptor cells.[71,72] A relationship between the elevation of cyclic GMP and photoreceptor cell degeneration has been established in normal eye rudiments of *Xenopus laevis* cultured in the presence of phosphodiesterase inhibitors.[73] Similarly, an abnormality in retinal cyclic GMP metabolism has been demonstrated in the inherited rod-cone dysplasia of Irish setters, due to a deficiency in cyclic GMP phosphodiesterase activity in affected visual cells.[74] Calmodulin, the protein activator of phosphodiesterase, may be involved in the canine disease, because the level of calmodulin in affected retinas is about one-half that in control retinas. Moreover, in contrast to normal dogs which develop a calmodulin independent phosphodiesterase with rod outer segment elongation, the affected dogs fail to develop evidence of this enzyme.[75,76]

Both phosphodiesterase activators[77] and inhibitors[78] are present in high concentrations in the retina. Study of these factors and delineation of their role in photoreceptor metabolism could provide a much better idea of normal and pathological processes in the photoreceptors.

The findings in the rd mouse and the Irish setter with inherited retinal degenerations have raised the possibility that accumulation of cyclic GMP may be a causative factor in photoreceptor cell degeneration. Recent studies have demonstrated that when phosphodiesterase inhibitors or dibutyryl cyclic GMP are applied to normal human retinas postmortem changes develop in rod photoreceptors in the absence of pronounced changes in the inner retina or loss of cones.[79] It has not been demonstrated that cyclic GMP changes lead to hereditary retinal degenerations in man; nevertheless, this line of investigation shows promise of delineating pathogenetic events, whether they be primary or secondary, which lead to photoreceptor cell death.

In the Royal College of Surgeons (RCS) rat with inherited retinal degeneration, a defect exists in the capacity of the pigment epithelium to phagocytize the rod outer segment tips. Studies of rat chimeras, produced from fusion of the embryos of normals and RCS mutants, have clearly established that the primary defect must be in the pigment epithelium, even though it is the adjacent photoreceptor cells that degenerate.[80] The defect in the pigment epithelium also has been demonstrated in vitro: pigment epithelial cells of RCS rats show marked reduction in the capacity to phagocytize outer segments when compared with the phagocytic capacity of normal rat pigment epithelial cells.[81] Factors that may regulate phagocytosis of outer segments, such as light,[82,83] are under study in animal models of hereditary retinal disease. Although phagosomes have been identified in human donor eyes with retinitis pigmentosa, a subtle quantitative defect in phagocytosis of outer segments has not yet been excluded.

Additional insights into possible pathogenetic mechanisms in human diseases are provided by other animal models of retinal degeneration. For example, a condition analogous to gyrate atrophy of the choroid and retina has been observed in one cat; this animal had hyperornithinemia and a deficiency of ornithine ketoacid transaminase in association with a generalized retinal degeneration involving photoreceptors, pigment epithelium, and small choroidal vessels.[84] Progress made in defining the ultrastructural abnormalities in the retinas of English setters with inherited neuronal ceroid lipofuscinosis[9] may prove relevant to humans with Batten-Mayou disease. Mutant mice with neurologic and retinal abnormalities have also provided new infor-

mation on pathogenetic mechanisms that may be involved in diseases of the human retina. [85]

Aids for the Visually Handicapped

A night vision pocketscope incorporating electrooptical technology has allowed patients with moderately advanced retinitis pigmentosa with defective receptor function to use their impaired cones to achieve mobility under dimly lit conditions. [86] The pocketscope does not alter the course of their condition, but it enables these patients to extend their daylight vision capabilities to nighttime. Other aids that have enhanced the ability of the partially sighted and blind to achieve more independence include talking calculators and typewriters as well as the Kurzweil reading machine (see *Volume Two, Part Six, Report of the Panel on Visual Impairment and Its Rehabilitation).*

Further Applications of Noninvasive Tests of Retinal Function

Noninvasive tests of retinal function have provided information on the site of defective function in a number of retinal disorders. For example, in Oguchi's disease, an inherited form of stationary night blindness, rhodopsin regeneration as measured by fundus reflectometry was normal at a time when the patient had abnormal rod psychophysical function. These and other findings suggested that the visual pigment cycle is normal in Oguchi's disease, but that visual function is abnormal due to a defect in neural adaptation in the inner retina proximal to the photoreceptors. [87]

In contrast, patients with fundus albipunctatus, another form of stationary night blindness, have an abnormality in visual pigment regeneration, as measured with fundus reflectometry, suggesting that a defect exists at the interface of the photoreceptors and pigment epithelium. [88,89] In addition to fundus reflectometry, ERG and psychophysical tests have also aided in defining the extent of cone relative to rod malfunction and the regions of retina that are involved. [90–92] In one form of dominant retinitis pigmentosa, foveal cone ERGs were normal in amplitude and implicit time at a time when midperipheral cone ERGs were minimally reduced but substantially delayed. These findings point to regional differences that exist in the cone involvement in the early stages of these diseases. [93]

Noninvasive tests also have helped to define pathogenetic mechanisms. For example, measurements with fundus reflectometry have shown that for the same reduction in rhodopsin content in a given peripheral retinal area, a patient with vitamin A deficiency showed an elevation of the dark adaptation threshold that was several orders of magnitude above that observed for patients with retinitis pigmentosa. [58] This finding and the results of ERG testing [94] support the idea that patients with retinitis pigmentosa with night blindness are not suffering from a local deficiency of vitamin A in the retina.

Other Advances

Research has continued on nutritionally induced retinal degeneration in the taurine-deficient cat, because this animal model provides an opportunity for studying photoreceptor malfunction that can be reversed by the reintroduction of taurine into the diet. [95,96] The mechanism by which taurine deficiency leads to photoreceptor cell death remains to be defined. Research has also proceeded on determining safe limits for light exposure, [97] for it is clear that prolonged exposure to high intensity lights can produce photoreceptor cell damage; [97,98] short wavelength light may be particularly harmful. [99]

These observations and an earlier report that photoreceptor degeneration is significantly retarded in albino RCS rats raised in darkness [100] led to a therapeutic trial on the effects of light deprivation. In two patients with hereditary retinitis pigmentosa, one eye was occluded by an opaque scleral contact lens worn for 6 to 8 hours a day over a five-year period. Despite this degree of monocular light deprivation, both retinas degenerated in a comparable manner, as judged by psychophysical and electroretinographic testing as well as ophthalmoscopic observations. [101]

Until more is known about the effects of light in such eyes, patients with retinitis pigmentosa and other hereditary retinal degenerations are advised to wear dark sunglasses for outdoor use. It must be emphasized, however, that no evidence at present demonstrates that any form of light deprivation or exposure will modify the rates at which these diseases progress. [101]

Diets deficient in vitamin E enhance the accumulation of lipofuscin in the retinal pigment epithelium, [102–104] and this may suggest an important line of investigation in hereditary retinal diseases in which lipofuscin-like material has been noted in high concentrations in the pigment epithelium. [105,106] Lymphocytes of patients with retinitis pigmentosa were stimulated by incubation with human soluble retinal antigens and with bovine rod outer segments, [107] thus suggesting that the cell-mediated immune system could be involved in the pathogenesis of these diseases.

Careful study of individual patients has been valuable in delineating disorders of the retina and choroid. This was demonstrated in the study of Refsum's disease, in which apparently unrelated clinical findings were recognized as firmly associat-

ed.[45] Once the constellation of clinical findings had been identified, accumulation of phytanic acid was detected in well-classified patients.[47] Gyrate atrophy was distinguished from other retinal degenerations clinically;[31] biochemical studies then substantiated the difference[32] and demonstrated heterogeneity among patients.[34,36,37]

Oculocutaneous albinism, inherited by an autosomal recessive mode, has been subdivided into tyrosinase-positive and tyrosinase-negative types based on the presence or absence of tyrosinase activity in the patient's hair bulb. Although children with both types are depigmented at birth, the tyrosinase-positive infant shows a slow increase in pigmentation and can be expected to have significantly better visual acuity than the tyrosinase negative infant.[108]

There are other recent examples of continued efforts to classify patients carefully, as a step toward defining biochemical defects and seeking rational approaches to treatment. These include the subclassification of pigmentary retinopathies associated with hearing loss[109] and a consideration of risk factors for genetic typing and detection in the different types of retinitis pigmentosa.[110]

RESEARCH NEEDS AND OPPORTUNITIES

Continued studies of blood, urine, and cultured skin fibroblasts are needed to search for new biochemical defects and further explore existing defects. This approach, as demonstrated in gyrate atrophy, Refsum's disease, and hereditary a-beta-lipoproteinemia, should lead to rational approaches for therapy.

Electroretinographic and psychophysical testing as well as fundus reflectometry are noninvasive techniques that provide an opportunity to assess retinal function in the early stages of the disease process and thereby detect functional abnormalities in various types of hereditary retinal and choroidal disorders. Delays in the temporal aspects of the cone b-wave of the electroretinogram, observed in progressive forms of retinitis pigmentosa, should be studied further to determine to what extent the delays are related to abnormal rod-cone[111] or cone-cone interactions and whether the delays in the b-wave reflect changes in the temporal aspects of the a-wave generated by the photoreceptors. Patients with these diseases often report problems not only in dark adaptation but also in their ability to adapt to light; the light-adapting properties of the normal and diseased retina might be explored profitably with noninvasive techniques. Emphasis should be placed on regional differences that may exist in the retina, as assessed by focal tests of retinal function and on techniques that enable the experimenter to visualize the stimulus on the retinal area of interest. Patients with congenital achromatopsia and some forms of stationary night blindness should be evaluated for they provide unusual opportunities to assess rod and cone function in isolation.

It is important to define further the short-term (that is, over five years) natural histories of the various types of hereditary retinal and choroidal diseases during various periods of life; such information is prerequisite to considering treatment trials aimed at stabilizing these conditions. Natural histories should be determined by periodically evaluating patients with full-field and focal electroretinographic testing, psychophysical tests, fundus reflectometry, photography, and such other techniques as may be developed.

The need to develop better visual aids for the partially sighted is related to the need for knowledge of the types of retinal malfunction that occur in these patients at different stages of disease. For those who are totally blind or blind and deaf, aids are needed to help them achieve as much independence and productivity as possible (see *Volume Two, Part Six, Report of the Panel on Visual Impairment and Its Rehabilitation*).

Studies of human donor eyes with retinal degenerations should be encouraged and correlation of premortem functional changes with postmortem findings emphasized. Donor eyes may be used not only for ultrastructural studies, but also biochemical studies of the retina and pigment epithelium. Attempts to establish cell lines of pigment epithelium for biochemical and ultrastructural analyses should be carried out first in normal donor eyes and where possible in postmortem donor eyes from patients with hereditary retinal diseases.

Animal models of hereditary retinal degenerations should continue to be studied (with particular attention given to defining the natural histories of these conditions) with noninvasive techniques; the associated metabolic and ultrastructural abnormalities should also be investigated. Treatment trials should be considered wherever rational programs can be started. Breeding facilities must continue to be maintained so that animal models are available for investigators who wish to study them (see Chapter 14, "Tissue Acquisition and Distribution: Human Donor Eyes and Animal Models"). The search for nonhuman primate models with hereditary retinal disease should be continued, because these models may provide new information on pathogenetic processes in human retinal degenerations.

Continued research on the normal retina and choroid must be encouraged, including studies of photoreceptor differentiation, interrelationships among retinal cells and between the photoreceptors and pigment epithelium, membrane architecture,

physical properties of cellular membranes, metabolic processes, specific cell types in tissue culture, postmortem metabolic capacity of the normal human retina and pigment epithelium, regional differences in the retina and pigment epithelium, and prenatal development of the retina and pigment epithelium, as well as postnatal changes with age. Information derived from studies of the normal retina and choroid should provide a basis for considering abnormalities in animals with retinal degenerations and in donor eyes from patients with hereditary retinal degenerations.

Continued efforts to detect carriers should be encouraged, whether through direct measurements of retinal function with electroretinographic or psychophysical testing, fundus reflectometry, or biochemical studies of blood, urine, and cultured skin fibroblasts. Where possible, prenatal tests for hereditary retinal and choroidal diseases also should be developed.

Epidemiological studies to search for factors that aggravate or ameliorate hereditary retinal regenerations should be encouraged based on evaluation of patients with well-classified disease. These studies should consider the drugs taken by the patient, the individual's nutritional status, and possible environmental factors.

When the rationale can be made, treatment trials should be encouraged for patients with retinal and choroidal degenerations that include appropriate assessment of visual function prior to the onset of treatment and throughout the course of the study.

Studies are needed to localize the genetic defects on specific chromosomal sites; these could lead to further definition of the biochemical defects in these diseases, detection of carriers, and development of prenatal tests to identify affected individuals.

The rapidly expanding field of molecular biology presents new possibilities for studying hereditary retinal and choroidal degenerations as well as learning more about basic retinal function. It is now possible to clone specific fragments of DNA and reintroduce them into the genome for copying. Most often this is performed using a viral vector, that is, linking the particular DNA fragment to viral DNA and introducing it into a cell. These techniques recently have been successful in producing such important proteins as insulin and interferon in cultured cell lines.[112] The first part of the procedure, that is, procuring cDNA copies of pertinent mRNA species potentially could yield much needed information on the biochemical differences in gene structure and function in normal and degenerative retinas. With these techniques, such basic questions as the following can be addressed now: Do genetic areas for rhodopsin coding differ in retinas with retinitis pigmentosa? Are differences observed in coding for phosphodiesterase, calmodulin, and other molecules?

The investigations proposed above require the efforts and cooperation of ophthalmologists, pathologists, optometrists, biochemists, cell biologists, immunologists, electrophysiologists, psychophysicists, anatomists, epidemiologists, statisticians, geneticists, and others with a knowledge and interest in the retina. Additional training is needed which provides the clinician with a better understanding of and appreciation for basic research approaches. At the same time, there is a need to familiarize nonclinician visual scientists with the research problems and opportunities presented by the hereditary retinal and choroidal diseases. Finally, there is continuing need for close collaborations among pathologists and visual scientists to ensure the availability and optimum investigation of human and animal material.

RECOMMENDATIONS

Based on the foregoing assessment of recent accomplishments, current activities, and research needs and opportunities in "Developmental and Hereditary Disorders," the Panel has made the following recommendations concerning research in this subprogram over the next five years. These have been grouped under two headings: Program Base and Program Development Priorities.

The Program Base includes areas of ongoing research where the current level of activity is considered adequate, or areas of ongoing research in which there may be great need for additional activity, but where, in the Panel's judgment, little or no opportunity (new methods or insights) exists at present to justify a significant expansion of effort. Nonetheless, additional applications for research grants in these areas may be funded if they are innovative and of very high quality as determined by the NIH peer review system.

Program Development Priorities include areas of ongoing research in which new knowledge and techniques offer particular opportunities for scientific progress, or promising new areas of research in which there is little or no support at present but where there is both great need and high potential for success. Such areas are judged to warrant significantly increased support over the next five years, provided that high quality applications for research grants in these areas are forthcoming.

Program Base

- Study animal models of developmental and hereditary disorders of the retina and choroid to define biochemical defects and pathogenetic mechanisms.

- Define the types of retinal malfunction and the short-term natural histories of these diseases using noninvasive techniques, including electroretinographic, psychophysical, and fundus reflectrometric testing (see Chapter 10, "Retinal Organization, Neurotransmission, and Adaptation;" and Chapter 13, "Noninvasive Techniques in the Study of Retinal Disorders").

Program Development Priorities

- Search for biochemical defects and pathogenetic mechanisms through studies of tissues, fluids, and postmortem donor eyes from patients with degenerative diseases of the retina and choroid. This will require basic research on normal humans to establish baseline data and clinicopathologic correlations combining premortem evaluations with postmortem histopathologic, biochemical, and other studies.
- Apply the powerful new methods of genetic analysis to studies of patients and animal models of hereditary retinal degenerations.
- Develop methods to detect carriers of these diseases and prenatal tests to identify affected individuals.
- Conduct epidemiologic studies on well-classified developmental and hereditary retinal and choroidal diseases to identify aggravating, ameliorating, and possible causative factors.
- Support therapeutic trials for degenerative diseases of the retina and choroid as promising new treatments become available.
- Evaluate the efficacy of new aids for the partially sighted (see *Volume Two, Part Six, Report of the Panel on Visual Impairment and Its Rehabilitation*).

RESOURCE REQUIREMENTS

After reviewing current research grant support in each of these categories and assessing the need and potential for future development, the Panel has estimated the number of projects it believes are needed to carry out its recommendations in FY 1983. These estimates are shown in the table on the following page. For a discussion of the general basis and significance of these projections, see the "Summary" at the beginning of this report.

RESOURCE TABLE

DEVELOPMENTAL AND HEREDITARY DISORDERS

	No. of Grants FY 1981	Panel Recommendation FY 83	
		Add. Grants	Total Grants
Program Base			
A. Study animal models of these diseases to define biochemical defects and pathogenetic mechanisms.	27	2	29
B. Define types of retinal malfunction and the short-term natural histories of these diseases using noninvasive techniques.	8	2	10
Program Development Priorities			
A. Search for biochemical defects and pathogenetic mechanisms through studies of patients with degenerative diseases.	7	3	10
B. Apply new methods of genetic analysis to studies of patients and animal models of hereditary retinal degenerations.	0	3	3
C. Develop methods to detect carriers of these diseases and prenatal tests to identify affected individuals.	0	2	2
D. Conduct epidemiologic studies on developmental and hereditary diseases of the retina and choroid.	0	3	3
E. Support therapeutic trials for degenerative diseases of the retina and choroid.	0	2	2
F. Evaluate the efficacy of new aids for the partially sighted.	*	*	*
Subtotal Grants	**42**	**17**	**59**
(% of Program)	**(11)**	**(15)**	**(12)**
Total Estimated Cost	**$3,809,000**	**$2,386,000**	**$6,195,000**

*See *Volume Two, Part Six, Report of the Visual Impairment and Its Rehabilitation* Panel.

REFERENCES

1. *Vision Problems in the U.S.* New York, National Society to Prevent Blindness, 1980.
2. Berson EL: Retinitis pigmentosa and allied retinal diseases: Electrophysiological findings. *Trans Am Acad Ophthalmol Otolaryngol* 81:659–666, 1976.
3. Boughman JA, Conneally PM, Nance WE: Population genetic studies of retinitis pigmentosa. *Am J Med Genet* 32:223–235, 1980.
4. LaVail MM, Sidman RL: C57BL/6J mice with inherited retinal degeneration. *Arch Ophthalmol* 91:394–400, 1974.
5. Van Nie R, Ivanyi D, Demant P: A new H-2-linked mutation, rds, causing retinal degeneration in the mouse. *Tissue Antigens* 12:106–108, 1978.
6. Bok D, Hall MO: The role of the pigment epithelium in the etiology of inherited retinal dystrophy in the rat. *J Cell Biol* 49:664–682, 1971.
7. Lai YL, Jones AM: Rat model for hereditary retinal degeneration, in Landers MB III, et al (eds): *Retinitis Pigmentosa*. New York, Plenum Publishing Corp, 1976, pp 115–136.
8. Buyukmichi N, Aguirre G, Marshall J: Retinal degenerations in the dog: II. Development of the retina in rod-cone dysplasia. *Exp Eye Res* 30:575–591, 1980.
9. Neville H, Armstrong D, Wilson B, et al: Studies on retina and pigment epithelium in hereditary canine ceroid lipofuscinosis: III. Morphologic abnormalities in retinal neurons and retinal pigmented epithelial cells. *Invest Ophthalmol Vis Sci* 19:75–86, 1980.
10. Berson EL, Watson G: Electroretinograms in English setters with neuronal ceroid lipofuscinosis. *Invest Ophthalmol Vis Sci* 19:87–90, 1980.
11. Aguirre GD: Inherited retinal degenerations in the dog. *Trans Am Acad Ophthalmol Otolaryngol* 81:667–676, 1976.
12. Aguirre G, Rubin LF: The electroretinogram in dogs with inherited cone degeneration. *Invest Ophthalmol Vis Sci* 14:840–847, 1975.
13. Fite KV, Boissy R, Smyth JR, et al: Neuroanatomical correlates of inherited retinal dystrophy in the amelanotic chicken. *Invest Ophthalmol Vis Sci* 20(suppl):41, 1981.
14. Santos-Anderson RM, Tso MOM, Wolf ED: Inherited retinopathy in collies: Light and electron microscopic study. *Invest Ophthalmol Vis Sci* 19:1281–1294, 1980.
15. Vainisi SG, Beck BB, Apple DJ: Retinal degeneration in a baboon. *Am J Ophthalmol* 78:279–284, 1974.
16. El-Mofty AAM, Eisner G, Balazs EA, et al: Retinal degeneration in rhesus monkeys, *Macaca mulatta*: Survey of three seminatural free-breeding colonies. *Exp Eye Res* 31:147–166, 1980.
17. Goodman G, Gunkel RD: Familial electroretinographic and adaptometric studies in retinitis pigmentosa. *Am J Ophthalmol* 46:142–178, 1958.
18. Gouras P, Carr RE: Electrophysiological studies in retinitis pigmentosa. *Arch Ophthalmol* 72:104–110, 1964.
19. Berson EL: Retinitis pigmentosa and allied diseases: Applications of electroretinographic testing, in Junk W (ed): *Int Ophthalmol.* The Hague 4:7–22, 1981.
20. Berson EL, Simonoff EA: Dominant retinitis pigmentosa with reduced penetrance: Further studies of the electroretinogram. *Arch Ophthalmol* 97:1286–1291, 1979.
21. Gouras P, Carr RE, Gunkel RD: Retinitis pigmentosa in a-beta-lipoproteinemia: Effects of vitamin A. *Invest Ophthalmol Vis Sci* 10:784–793, 1971.
22. Berson EL, Rosen JB, Simonoff EA: Electroretinographic testing as an aid in detection of carriers of X-chromosome-linked retinitis pigmentosa. *Am J Ophthalmol* 87:460–468, 1979.
23. Berson EL, Goldstein EB: The early receptor potential in sex-linked retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 9:58–63, 1970.
24. Highman VN, Weale RA: Rhodopsin density and visual threshold in retinitis pigmentosa. *Am J Ophthalmol* 75:822–832, 1973.
25. Gieser DK, Fishman GA, Cunha-Vaz J: X-linked recessive retinitis pigmentosa and vitreous fluorophotometry: A study of female heterozygotes. *Arch Ophthalmol* 98:307–310, 1980.
26. Berson EL: Retinitis pigmentosa and allied diseases: Early diagnosis and some aspects of prevention. Presented at the National Society to Prevent Blindness, New York, October 1980.
27. Valle DL, Kaiser-Kupfer M, Del Valle LA: Gyrate atrophy of the choroid and retina: Deficiency of ornithine aminotransferase in transformed lymphocytes. *Proc Natl Acad Sci USA* 74:5159–5161, 1977.
28. Kennaway NG, Weleber RG, Buist NRM: Gyrate atrophy of choroid and retina: Deficient activity of ornithine ketoacid aminotransferase in cultured skin fibroblasts. *N Engl J Med* 297:1180, 1977.
29. Shih VE, Berson EL, Mandell R, et al: Ornithine ketoacid transaminase deficiency in gyrate atrophy of the choroid and retina. *Am J Hum Genet* 30:174–179, 1978.
30. Brady RO: Ophthalmologic aspects of lipid storage diseases. *Trans Am Acad Ophthalmol Otolaryngol* 85:1007–1013, 1978.
31. Fuchs E: Euber zwei der Retinitis pigmentosa verwandte Krankheiten (Retinitis punctate albescens und Atrophia gyrata chorioideae et retinae). *Arch Augenheilk* 32:111, 1896.
32. Takki K, Simell O: Genetic aspects in gyrate atrophy of the choroid and retina with hyperornithinaemia. *Br J Ophthalmol* 58:907–916, 1974.
33. Trijbels JBF, Sengers RCA, Bakkeren JAJM, et al: L-ornithine-ketoacid-transaminase deficiency in cultured fibroblasts of a patient with hyperornithinemia and gyrate atrophy of the choroid and retina. *Clin Chim Acta* 79:371, 1977.
34. Berson EL, Schmidt SY, Shih VE: Ocular and biochemical abnormalities in gyrate atrophy of the choroid and retina. *Ophthalmology* 85:1018–1027, 1978.
35. O'Donnell JJ, Sandman RP, Martin SR: Gyrate atrophy of the retina: Inborn error of L-ornithine: 2-oxoacid aminotransferase. *Science* 200:200–201, 1978.

36. Weleber RG, Kennaway NG, Buist NRM: Vitamin B^6 in management of gyrate atrophy of choroid and retina. *Lancet* 2:1213, 1978.
37. Kaiser-Kupfer MI, Valle D, Bron AJ: Clinical and biochemical heterogeneity in gyrate atrophy. *Am J Ophthalmol* 89:219–222, 1980.
38. Valle D, Walser M, Brusilow SW, et al: Gyrate atrophy of the choroid and retina: Amino acid metabolism and correction of hyperornithinemia with an arginine-deficient diet. *J Clin Invest* 65:371–378, 1980.
39. Kuwabara T, Ishikawa Y, Kaiser-Kupfer MI: Experimental models of gyrate atrophy in animals. *Ophthalmology* 88:331–334, 1981.
40. Berson EL, Shih VE, Sullivan PL: Ocular findings in patients with gyrate atrophy on pyridoxine and low-protein, low arginine diets. *Ophthalmology* 88:311–315, 1981.
41. Kaiser-Kupfer MI, de Monasterio FM, Valle D, et al: Gyrate atrophy of the choroid and retina: Improved visual function following reduction of plasma ornithine by diet. *Science* 210:1128–1131, 1980.
42. Kaiser-Kupfer MI, de Monasterio FM, Valle D, et al: Visual results of a long-term trial of a low-arginine diet in gyrate atrophy of choroid and retina. *Ophthalmology* 88:307–310, 1981.
43. McInness RR, Arshinoff SA, Bell L, et al: Hyperornithinaemia and gyrate atrophy of the retina: Improvement of vision during treatment with a low-arginine diet. *Lancet* 1:513–517, 1981.
44. Takki K, Milton RC: The natural history of gyrate atrophy of the choroid and retina. *Ophthalmology* 88:292–301, 1981.
45. Refsum S: Heredopathia atactica polyneuritiformis: A familial syndrome not hitherto described. *Acta Psychiatr Neurol Scand Suppl* 38:1–303, 1946.
46. Refsum S: Heredopathia atactica polyneuritiformis: Phytanic storage disease, Refsum's disease: A biochemically well-defined disease with a specific dietary treatment. *Arch Neurol* 38:605–606, 1981.
47. Klenk E, Kahlke W: Uber das Vorkommen der 3,7,11,15-Tetramethyl-hexadecansaure (Phytansaure) in den Cholesterinestern und anderen Lipoidfraktionen der Organe bei einem Krankheitsfall unbekannter Genese (Verdacht auf Heredopathia atactica polyneuritiformis (Refsum-Syndrome). *Hoppe Seylers Z Physiol Chem* 333:133–139, 1963.
48. Steinberg D, Mize CE, Herndon JH Jr, et al: Phytanic acid in patients with Refsum's syndrome and response to dietary treatment. *Arch Intern Med* 125:75–87, 1970.
49. Eldjarn L, et al: Dietary effects on serum phytanic-acid levels and on clinical manifestation in heredopathia atactia polyneuritiformis. *Lancet* 1:691–693, 1966.
50. Masters-Thomas A, Bailes J, Billimoria JD, et al: Heredopathia atactica polyneuritiformis (Refsum's disease): I. Clinical features and dietary management. *J Hum Nutr* 34:245–250, 1980.
51. Brady RO: Heritable catabolic and anabolic disorders of lipid metabolism. *Metabolism* 26:329–345, 1977.
52. Johnson WG, Desnick RJ, Long DM, et al: Intravenous injection of purified hexoseaminidase A into a patient with Tay-Sachs disease, in Desnick RJ, Bernlohr RW, Krivit W (eds): *Enzyme Therapy in Genetic Diseases*. New York, National Foundation, 1973, pp 120–124.
53. Barranger JA, Pentchev PG, Rapoport SI, et al: Augmentation of brain lysosomal enzyme activity following enzyme infusion with concomitant alterations of the blood brain barrier. *Trans Am Neurol Assoc* 102:10–12, 1977.
54. Kolb H, Gouras P: Electron microscopic observations of human retinitis pigmentosa, dominantly inherited. *Invest Ophthalmol Vis Sci* 13:487–498, 1974.
55. Szamier RB, Berson EL: Retinal ultrastructure in advanced retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 16:947–962, 1977.
56. Szamier RB, Berson EL, Klein R, et al: Sex-linked retinitis pigmentosa: Ultrastructure of photoreceptors and pigment epithelium. *Invest Ophthalmol Vis Sci* 18:145–160, 1979.
57. Szamier RB: Ultrastructure of the preretinal membrane in retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 21:227–236, 1981.
58. Ripps H, Brin KP, Weale RA: Rhodopsin and visual threshold in retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 17:735–745, 1978.
59. Schmidt SY, Berson EL: Postmortem metabolic capacity of photoreceptor cells in human and rat retina. *Invest Ophthalmol Vis Sci* 19:1274–1280, 1980.
60. Mannagh J, Arya DV, Irvine AR Jr: Tissue culture of human retinal pigment epithelium. *Invest Ophthalmol Vis Sci* 12:52–64, 1973.
61. Edwards RB: Culture of mammalian retinal pigment epithelium and neural retina, in Packer L (ed): *Methods of Enzymology*, Vol 81, Biomembranes, part H. New York, Academic Press, 1982, pp 39–43.
62. Edwards RB: Glycosaminoglycan synthesis by cultured human retinal pigment epithelium from normal post-mortem donors and a post-mortem donor with retinitis pigmentosa. *Invest Ophthalmol Vis Sci*, in press.
63. Haley JF, Flood MT, Gouras P: Changes of protein patterns in human retinal pigment epithelial cells in vitro. *Invest Ophthalmol Vis Sci* 20(suppl):165, 1981.
64. Flood MT, Gouras P, Kjeldbye H: Growth characteristics and ultrastructure of human retinal pigment epithelium in vitro. *Invest Ophthalmol Vis Sci* 19:1309–1320, 1980.
65. Israel P, Masterson E, Goldman A, et al: Retinal pigment epithelial cell differentiation in vitro. *Invest Ophthalmol Vis Sci* 19:720–727, 1980.
66. Feeney L: Lipofuscin and melanin of human retinal pigment epithelium: Fluorescence, enzyme cytochemical, and ultrastructural studies. *Invest Ophthalmol Vis Sci* 17:583–600, 1978.
67. Feeney-Burns L, Berman ER, Rothman H: Lipofuscin of human retinal pigment epithelium. *Am J Ophthalmol* 90:783–791, 1980.
68. Peisch RD, Schmidt SY: Variations in melanin concentration in the pigment epithelium of postmortem human eyes. *Invest Ophthalmol Vis Sci* 18(suppl):20, 1979.

69. Newsome DA, Fletcher RT, Chader GJ: Cyclic nucleotides vary by area in retina and pigmented epithelium of human and monkey. *Invest Ophthalmol Vis Sci* 19:864–869, 1980.
70. Schmidt SY, Lolley RN: Cyclic nucleotide phosphodiesterase: An early defect in inherited retinal degeneration of C3H mice. *J Cell Biol* 57:117–123, 1973.
71. Farber DB, Lolley RN: Cyclic guanosine monophosphate: Elevation in degenerating photoreceptor cells of the C3H mouse retina. *Science* 186:449–451, 1974.
72. Farber DB, Lolley RN: Enzymic basis for cyclic GMP accumulation in degenerative photoreceptor cells of mouse retina. *J Cyclic Nucleotide Res* 2:139–148, 1976.
73. Lolley RN, Farber DB, Rayborn ME, et al: Cyclic GMP accumulation causes degeneration of photoreceptor cells: Simulation of an inherited disease. *Science* 196:664–666, 1977.
74. Aguirre G, Farber D, Lolley R, et al: Rod-cone dysplasia in Irish setters: A defect in cyclic GMP metabolism in visual cells. *Science* 201:1133–1134, 1978.
75. Liu YP, Krishna G, Aguirre GD, et al: Involvement of cyclic GMP phosphodiesterase activator in a hereditary retinal degeneration. *Nature* 280:62, 1979.
76. Chader GJ, Liu YP, Fletcher RT, et al: Cyclic GMP phosphodiesterase and calmodulin in early-onset inherited retinal degenerations, in Miller W (ed): *Current Topics in Membranes and Transport*, Vol 15. New York, Academic Press, 1981, pp 133–156.
77. Lin Y, Schwartz H: Protein activator of cyclic AMP phosphodiesterase and cyclic nucleotide phosphodiesterase in bovine retina and bovine lens. *Biochim Biophys Acta* 526:186–193, 1978.
78. Dumler I, Etingof R: Protein inhibitor of cyclic adenosine 3′:5′-monophosphate phosphodiesterase in retina. *Biochim Biophys Acta* 429:474–484, 1976.
79. Ulshafer RJ, Garcia CA, Hollyfield JG: Sensitivity of photoreceptors to elevated levels of cGMP in the human retina. *Invest Ophthalmol Vis Sci* 19:1236–1241, 1980.
80. Mullen RJ, LaVail MM: Inherited retinal dystrophy: primary defect in pigment epithelium determined with experimental rat chimeras. *Science* 192:799–801, 1976.
81. Edwards RB, Szamier RB: Defective phagocytosis of isolated rod outer segments by RCS rat retinal pigment epithelium in culture. *Science* 197:1001–1003, 1977.
82. LaVail MM: Rod outer segment disk shedding in rat retina: Relationship to cyclic lighting. *Science* 194:1071–1073, 1976.
83. Hall MO: Phagocytosis of light- and dark-adapted rod outer segments by cultured pigment epithelium. *Science* 202:526–528, 1978.
84. Valle DL, Boison AP, Jecyk P, et al: Gyrate atrophy of choroid and retina in a cat. *Invest Ophthalmol Vis Sci* 20:251–255, 1981.
85. LaVail MM: Analysis of neurological mutants with inherited retinal degeneration: Friedenwald Lecture. *Invest Ophthalmol Vis Sci* 21:638–657, 1981.
86. Berson EL, Rabin AR, Mehaffey L: Advances in night vision technology: A pocketscope for patients with retinitis pigmentosa. *Arch Ophthalmol* 90:427–431, 1973.
87. Carr RE, Ripps H, Siegel IM, et al: Rhodopsin and the electrical activity of the retina in congenital night blindness. *Invest Ophthalmol Vis Sci* 5:497–507, 1966.
88. Ripps H: Night blindness and the retinal mechanisms of visual adaptation. *Ann R Coll Surg Engl* 58:2, 1976.
89. Carr RE, Ripps H, Siegel IM: Visual pigment kinetics and adaptation in fundus albipunctatus. *Doc Ophthalmol Proceeding Series XI ISCERG Symposium*, Bad Tauheim, West Germany, Junk W bv, The Hague, pp 193–204, 1974.
90. Massof RW, Finkelstein D: Rod sensitivity relative to cone sensitivity in retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 18:263–272, 1979.
91. Young RSL, Fishman GA: Color matches of patients with retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 19:967–972, 1980.
92. Sandberg MA, Jacobson SG, Berson EL: Foveal cone electroretinograms in retinitis pigmentosa and juvenile macular degeneration. *Am J Ophthalmol* 88:702–707, 1979.
93. Sandberg MA, Effron MH, Berson EL: Focal cone electroretinograms in dominant retinitis pigmentosa with reduced penetrance. *Invest Ophthalmol Vis Sci* 17:1096–1101, 1978.
94. Sandberg MA, Rosen JB, Berson EL: Cone and rod function in vitamin A deficiency with chronic alcoholism and in retinitis pigmentosa. *Am J Ophthalmol* 84:658–665, 1977.
95. Berson EL, Hayes KC, Rabin AR, et al: Retinal degeneration in cats fed casein: II. Supplementation with methionine, cysteine or taurine. *Invest Ophthalmol Vis Sci* 15:52–58, 1976.
96. Schmidt SY, Berson EL, Watson G, et al: Retinal degeneration in cats fed casein: III. Taurine deficiency and ERG amplitudes. *Invest Ophthalmol Vis Sci* 16:673–678, 1977.
97. Sperling HG: Intense light hazards in ophthalmic diagnosis and treatment: Proceedings of a Symposium, Houston, Texas, October 25–26, 1979. *Vis Res* 20:1033–1203, 1980.
98. Sperling HG: Are ophthalmologists exposing their patients to dangerous light levels? *Invest Ophthalmol Vis Sci* 19:989–990, 1980.
99. Ham WT Jr, Ruffolo JJ Jr, Mueller HA, et al: Histologic analysis of photochemical lesions produced in rhesus retina by short wavelength light. *Invest Ophthalmol Vis Sci* 17:1029–1035, 1978.
100. Dowling JE, Sidman RL: Inherited retinal dystrophy in rats. *J Cell Biol* 14:73–109, 1962.
101. Berson EL: Light deprivation and retinitis pigmentosa. *Vision Res* 20:1179–1184, 1980.
102. Robison WA, Kuwabara T, Bieri JA: Vitamin E deficiency and the retina: Photoreceptor and pigment epithelial changes. *Invest Ophthalmol Vis Sci* 18:683–690, 1979.
103. Hayes KC: Retinal degeneration in monkeys induced by deficiencies of vitamin E or A. *Invest Ophthalmol Vis Sci* 13:499–510, 1974.

104. Katz ML, Stone WL, Dratz EA: Fluorescent pigment accumulation in retinal pigment epithelium of antioxidant-deficient rats. *Invest Ophthalmol Vis Sci* 17:1049–1058, 1978.
105. Miller SA: Fluorescence in Best's vitelliform dystrophy, lipofuscin, and fundus flavimaculatus. *Br J Ophthalmol* 62:256, 1978.
106. Eagle RC Jr, Lucier AC, Bernardino VB Jr, et al: Retinal pigment epithelial abnormalities in fundus flavimaculatus: A light and electron microscopic study. *Ophthalmology* 87:1189–1200, 1980.
107. Brinkman CJJ, Pinckers AJLG, Broekhuyse RM: Immune reactivity to different retinal antigens in patients suffering from retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 19:743–750, 1980.
108. Siegel IM: Ophthalmological findings in tyrosinase positive oculocutaneous albinism. *Perspect Ophthalmol* 3:17–23, 1979.
109. Bateman JB, Riedner ED, Levin LS, et al: Heterogeneity of retinal degeneration and hearing impairment syndromes. *Am J Ophthalmol* 90:755–767, 1980.
110. Berson EL, Rosner B, Simonoff EA: Risk factors for genetic typing and detection in retinitis pigmentosa. *Am J Ophthalmol* 89:763–775, 1980.
111. Sandberg MA, Berson EL, Effron MH: Rod-cone interaction in the distal human retina. *Science* 212:829–831, 1981.
112. Goeddel D, et al: The structure of eight distinct cloned human leukocyte interferon cDNA's. *Nature* 290:20–26, 1981.

5

MACULAR DEGENERATION

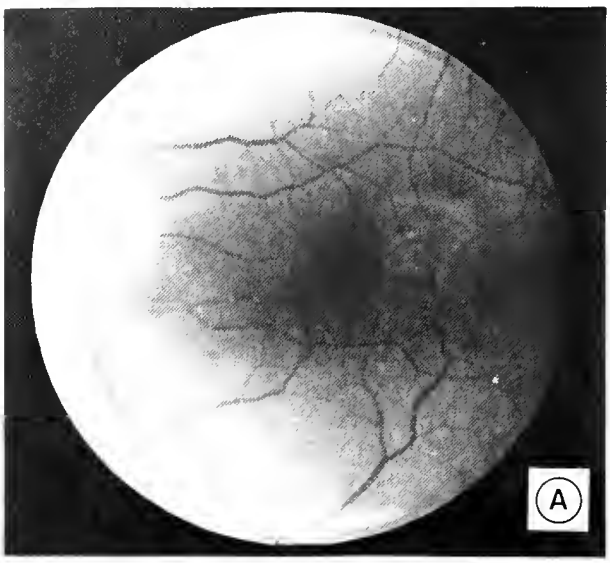
INTRODUCTION

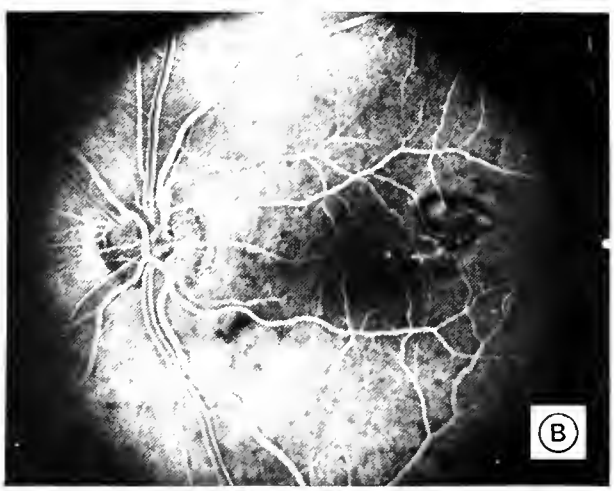
THE MACULA IS the tiny central part of the sensory retina, only one-fifth of an inch across (see Chapter 9, "Photoreceptors, Visual Pigments, and Phototransduction," and Chapter 10, "Retinal Organization, Neurotransmission, and Adaptation"). But our central vision and, therefore, the ability to read and to see fine detail depends on the health of this very small, highly specialized area of the retina. Unfortunately, the macular region of the retina is predisposed to various degenerative changes, and although there are a number of anatomical specializations within the macula, including a high density of modified cones and a central zone which is avascular, the underlying reason for this predisposition to disease is not known. According to 1970–1974 data from a national survey, in the United States each year more than 165,000 first visits to physicians are made because of macular degeneration.[1] For some, the disease is so severe it causes legal blindness. For many others, sharp, central vision is significantly impaired. Although macular diseases affect all age groups, most cases are associated with aging. For the affected individuals, the results can be tragic; for society, the economic consequences are enormous.

The term macular disease actually refers to a complex group of disorders which include:

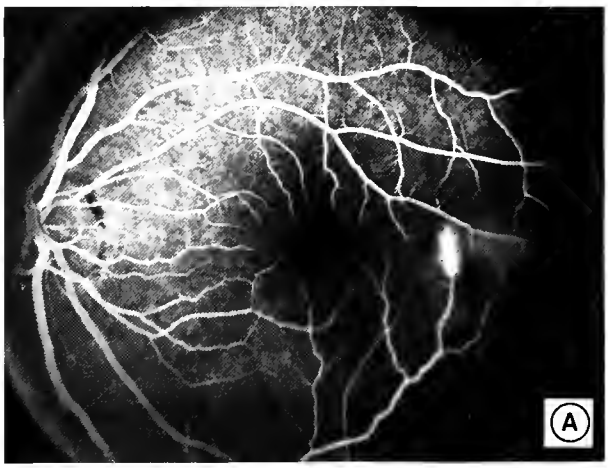
- **Aging-related maculopathy (senile macular degeneration).** (The term senile macular degeneration, although firmly established in ophthalmologic terminology, has unfortunate connotations. For this reason, the term aging-related maculopathy will be used in this chapter and elsewhere in this report.) This is a major cause of blindness in the United States, and by far the leading cause of new cases of blindness in people age 65 and over. The disease may progress slowly in some people. In others, however, vision may deteriorate quite rapidly. These are people with the neovascular form of the disease in which abnormal new blood vessels from the choroid, the blood vessel layer behind the retina, grow beneath and into the macula (Figure 1).
- **Central serous choroidopathy.** This is a disorder characterized by the accumulation of fluid beneath the retina and detachment of the retina in the macular region (Figure 2). Younger individuals age 20 to 40 are primarily affected. Vision is distorted and there may be repeated episodes. The degree of recovery is variable, and some individuals sustain permanent visual loss.
- **Hereditary (juvenile) macular degeneration.** This is a group of genetically transmitted degenerations of the macula affecting the young. The main types include vitelliform macular degeneration (Best's macular dystrophy) and fundus flavimaculatus (Stargardt's disease) (Figure 3).
- **Macular disease associated with other ocular or systemic conditions.** This category includes macular edema (swelling), which may occur following cataract surgery or retinal detachment surgery, or which may be associated with vascular or inflammatory disorders, and the macular degeneration that occurs in the presumed ocular histoplasmosis syndrome (Figure 4), trauma, and high degrees of myopia.
- **Toxic macular disease.** The central visual area has a special susceptibility to the toxic effects of several types of drugs and chemicals (see Chapter 7, "Toxic and Environmental Disorders").

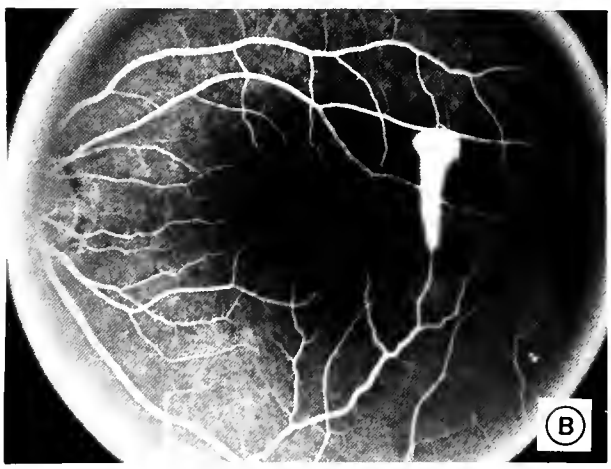
A significant breakthrough recently occurred in the ability to treat aging-related maculopathy. An NEI grant-supported clinical trial, the Macular Photocoagulation Study, determined that laser pho-





FIGURES 1A, B, and C. Increasing fluorescence from a subretinal neovascular membrane just superior and temporal to the foveal area.





FIGURES 2A and B. Leakage of fluorescein in smokestack configuration in a patient with central serous choroidopathy.

tocoagulation is effective in preventing severe visual loss from the neovascular type of this disease,[2] which accounts for 90 percent of blindness from aging-related maculopathy.[16] On the basis of the study's findings, it can be estimated that with prompt treatment 13,000 Americans with neovascular aging-related maculopathy each year could be permanently or temporarily saved from going blind, and 120,000 more spared lesser degrees of visual impairment.

As striking as this treatment breakthrough is, the results are limited to one specific type of macular disease, and then only to patients meeting stringent eligibility criteria. For the majority of people with aging-related maculopathy and for people affected by other macular diseases, there is no proven treatment. None of the fundamental causes of any type of macular disease is known, and none can be

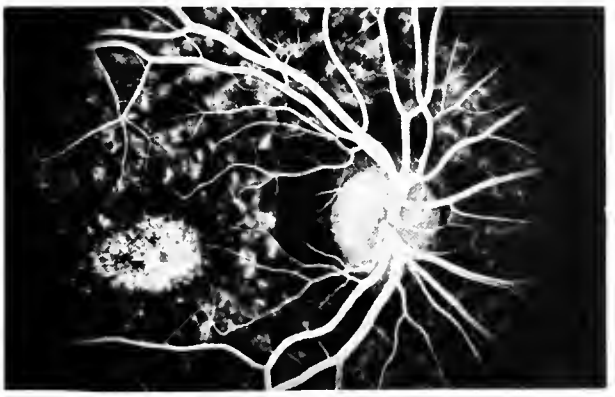


FIGURE 3. Dark fundus appearance with areas of fluorescein transmission in the fovea and posterior pole of a patient with Stargardt's Disease.

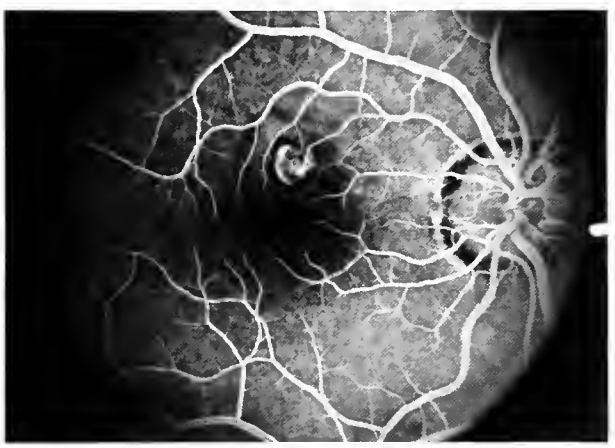


FIGURE 4. Subretinal neovascularization in a patient with presumed ocular histoplasmosis syndrome.

prevented. Therefore, research on macular diseases must take advantage of new opportunities for learning about basic disease mechanisms. Many of these opportunities are closely related to, and are derived from, other research on the retina, the retinal pigment epithelium, and the choroid (see Chapter 1, "Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities;" Chapter 8, "Retinal Pigment Epithelium;" and Chapter 9). Of particular importance is research on the metabolic and functional interrelationships of these tissues and the changes that occur during the aging process. Research on the mechanisms that control new blood vessel growth is another important cross-cutting area of investigation (see Chapter 1), as is research on the effects of age, light, and oxidative and other metabolic processes on the functional integrity of the macula (see Chapter 8 and Chapter 9).

SUBPROGRAM OBJECTIVES

- To understand the metabolic and functional interrelationships among the macula, the retinal pigment epithelium, and the choroid in the normal and diseased state.
- To determine the causes for subretinal neovascularization, macular degeneration, and macular edema.
- To determine the natural course of macular diseases, to improve diagnosis and treatment, and to find means of prevention.

OVERVIEW OF CURRENT RESEARCH SUPPORT

During FY 1981, the National Eye Institute funded 18 grants for research in macular disease at a total cost of $1,418,000. This included 13 grants (involving 12 clinics) for the conduct of the Macular Photocoagulation Study (MPS).

The objective of the MPS is to evaluate the usefulness of argon laser photocoagulation in the treatment of aging-related maculopathy and the presumed ocular histoplasmosis syndrome, and the documentation of the natural history of both diseases. Specifically, the study is intended to determine whether use of the laser to obliterate extrafoveal choroidal neovascular membranes preserves visual acuity in eyes affected by one or the other of the diseases. Recruitment of new patients in the Senile Macular Degeneration Study (SMDS) part of the MPS has recently been terminated because of a positive treatment effect (see Introduction and Accomplishments). Follow-up of all SMDS patients will continue in order to determine the long-term results. The natural history study and investigation of the presumed ocular histoplasmosis syndrome continue.

The remaining 8 grants in this subprogram were for basic research on macular edema, animal models of macular disease, and noninvasive evaluations of macular function.

Also of relevance to research in macular degeneration were 6 NEI grants in the Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular

Abnormalities subprogram that supported the Branch Vein Occlusion Study (see Chapter 1). This is an ongoing multicenter randomized controlled clinical trial with the objective of determining if laser photocoagulation is useful in the management of branch vein occlusion complications (neovascularization, vitreous hemorrhage, and macular edema).

RECENT ACCOMPLISHMENTS

The Macular Photocoagulation Study, begun in 1979, supports multicenter natural history studies and controlled clinical trials to test the efficacy of argon laser photocoagulation therapy in the treatment of two macular diseases: (1) aging-related maculopathy (Senile Macular Degneration Study) and (2) the presumed ocular histoplasmosis syndrome. Patient recruitment in the SMDS was terminated in the third year of the projected five year trial because, after 18 months of follow-up, 25 percent of treated eyes, compared to 60 percent of untreated eyes, had experienced severe visual loss.[2] Follow-up of all SMDS patients will continue in order to determine the long-term results of treatment.

The eligibility criteria for the SMDS included drusen, angiographic evidence of a chroroidal neovascular membrane 200 to 2,500 μm from the center of the foveal avascular zone, and a visual acuity of 20/100 or better. The SMDS results do not apply to the more common dry (drusenoid) form of the disease, nor to eyes with neovascular membranes within 200 μm of the center of the foveal avascular zone—these manifestations were not studied.

Based on these results, it can be estimated that over the next decade, with prompt laser treatment, 130,000 people with the neovascular form of aging-related maculopathy could be permanently or temporarily saved from going blind. And an estimated 1.2 million more may be spared lesser degrees of visual impairment.

Notable progress has also been made over the last several years in understanding the basic mechanisms of macular disease. Ultrastructural and tracer studies of the retinal pigment epithelium-Bruch's membrane-choriocapillaris complex indicate that a mechanical barrier to passive molecular transport exists at the level of the zonula occludens of the retinal pigment epithelium.[3] Serous detachments of retinal pigment epithelium and sensory retina have been produced in the rabbit by transcleral, subretinal injection of collagenase and hyaluronidase.[4] This technique has been adapted to primates in an effort to produce chemical and mechanical damage to Bruch's membrane or the retinal pigment epithelium in the macular region, allowing proliferation of choroidal neovascularization into the potential subretinal space created by serous detachment.[5]

A reproducible model of subretinal neovascularization, developed in the *Macacca speciosa* (stump-tail monkey), offers new opportunities for the study of disciform macular degeneration.[5] Ischemia, hemorrhage, locale (the macular region versus the periphery), and other factors which may relate to the development and evaluation of subretinal neovascularization can be evaluated with this model.

In addition, several free-ranging groups of rhesus monkeys (*Macacca mulatta*) have been studied to collect information on aging of the eye and the joints of a subhuman primate species.[6] In the course of this investigation, an interesting retinal degeneration was discovered in which two types of anomalies were noted: (1) a macular pigmentary disorder showing various degrees of irregularity in the distribution and intensity of pigmentation, for example, a stippling and mottling that resembles the pigment changes in human macular degeneration, and (2) multiple discrete small whitish spots, clustered mainly in the paramacular area but occurring occasionally within the macula and, less frequently, in the periphery.

Recently, through clinicopathologic correlation, it has been learned that the changes in Stargardt's disease are due to the accumulation of excess amounts of lipofuscin-like material in the retinal pigment epithelium.[7]

Ultrastructural studies of English setters with inherited ceroid lipofuscinosis have shown accumulation of abnormal cytosomes within neurons and retinal pigment epithelial cells.[8] These cellular abnormalities may be related to deficiencies of peroxidase and defects of lipid peroxidation, changes which are similar in many respects to those reported in humans with Batten's disease; thus, this animal may be a useful model of this disorder.

Pigment epithelial dystrophy in the dog apparently may be inherited as an autosomal dominant trait and offers another available animal model for the study of hereditary retinal degenerations.[9]

Malignant tumors can produce a substance that induces the formation of new blood vessels.[10] Investigators suspect that there may be a comparable mechanism in the production of retinal and choroidal neovascularization[11,12] (see Chapter 1).

Epidemiological studies of macular disease are gaining in importance as an avenue of investigation. Blindness registries,[13–15] case-control studies,[16–18] and population based studies[19–21] have provided some data concerning aging-related maculopathy. The Framingham Eye Study[19] found aging-related maculopathy to be more prevalent in women than in men, but a recent analysis of these data[22] and an analysis of data from the first National Health and

Nutrition Survey[23] indicate that women do not have an excess risk of developing aging-related maculopathy. Case-control studies have all shown an association between aging-related maculopathy and hyperopia.[16–18] A family history of aging-related maculopathy has been identified as a risk factor,[16] an observation that also has been made in a number of clinical reports. The Framingham Eye Study found a positive association between aging-related maculopathy and hypertension, history of prior lung infection and other variables. Because of the large number of variables (667) included in that study, however, it is likely that some of these associations are due to chance alone. Evidence for and against these possible associations has recently been reviewed.[24]

Preliminary epidemiologic studies have suggested that histoplasmosis may be the second leading cause of visual loss in people under the age of 50 who live in an endemic area in which some 75 million people reside. Patients with histoplasmosis usually lose visual acuity due to the consequences of a neovascular membrane in the macula.

RESEARCH NEEDS AND OPPORTUNITIES

Subretinal neovascularization and its disastrous sequelae (the disciform response) are a common denominator in many macular disease processes, including aging-related maculopathy, the presumed ocular histoplasmosis syndrome, angioid streaks, and high degrees of myopia. There is an urgent need to understand what causes and controls the abnormal growth of new choroidal blood vessels and, ultimately, to determine what can be done to prevent it from occurring. Although the aging process is implicated, the abnormalities in Bruch's membrane and the retinal pigment epithelium which enable blood vessels from the choroid to grow inward beneath the macula have no known cause. Biochemical and anatomic studies should be expanded to identify the changes occurring with age in this complex. There is a remarkable difference between white and black patients in the incidence of macular detachment from a wide variety of causes. Some effort should be made to determine whether there are structural or other changes in the macula that protect black people from macular detachment and choroidal neovascularization.

Information is needed about the basis of retinal pigment epithelium proliferation, abnormal collagen synthesis, and pigment migration which is frequently seen in macular disease. The role of the retinal pigment epithelium in the metabolism of Bruch's membrane, and the composition and mechanism for the formation of drusen, must be elucidated. Tissue culture systems for study of normal and diseased human retinal pigment epithelium should be improved and used to seek biochemical and metabolic defects.

Experimental animal models of macular disease, particularly primate models,[5,25] should continue to be developed. The evaluation of proposed factors and hypotheses for the genesis of subretinal neovascularization and the disciform response is but one area where the use of animal models could provide new routes of investigation.

The search for extracellular factors which modulate angiogenesis[11,12] (see Chapter 1) is another important area for investigation. Purification and characterization of these postulated factors should receive high priority as should testing of these substances in appropriate experimental models of subretinal neovascularization.

Epidemiological studies are needed of patients with aging-related maculopathy (and other macular diseases) and age, race, and sex-matched controls who do not have the disease are needed to identify possible risk factors for the disease. Such factors as hair and eye color, cell surface antigens (the HLA-B7 and DRW antigens and other markers), light exposure, occupational history, diet, and the history of other ocular and systemic disease might be revealing.[26,27] Biochemical studies could be initiated using skin biopsies for growth of fibroblast cells from aging-related maculopathy patients and controls.

Retinal pigment epithelium and retinal capillary epithelium barrier and directional transport properties are another area of opportunity (see Chapter 8). Studies are needed on the mechanisms for fluid transport across the retinal pigment epithelium and on the pharmacologic modulation of this transport. More information is needed about how retinal pigment epithelium tight junctions are formed and maintained and whether the striking asymmetries in the distribution of ionic pumps and surface membrane components are affected by macular disease processes. These studies would be directly relevant to understanding such clinical problems as rhegmatogenous detachment, secondary exudative detachment, central serous choroidopathy, cystoid macular edema, and other macular conditions.

The relationships between light, natural antioxidants (for example, selenium and vitamin E), lipofuscin, and aging should continue to be explored. Although speculative, this hypothesis for retinal light damage may offer a clue to the pathogenesis of macular degeneration and should be rigorously investigated (see Chapter 8 and Chapter 9).

The basis for the predisposition of the macular region to various degenerative changes is not at all clear. There are many anatomical specializations in the macular region, such as a very high density of

cones with elongated outer segments and a capillary-free area. These architectural modifications undoubtedly contribute to the acute vision characteristic of the primate macula. But what are the physiological and metabolic consequences of this specialization? A critical comparison of differences betwen the central and peripheral retina may provide clues to the susceptibility of the macular region to disease (see Chapter 8, Chapter 9, and Chapter 10). Additionally, the effect which the macular yellow pigment has on visual perception and its possible role in protecting the macula against photic damage need to be investigated.

Improved noninvasive psychophysical and electrophysiological tests of macular function are needed (see Chapter 4, "Developmental and Hereditary Disorders" and Chapter 13, "Noninvasive Techniques in the Study of Retinal Disorders") to improve the detection of early defects in macular vision, particularly those that are harbingers of the more destructive types of macular disease. Noninvasive tests of macular function should also be of value in evaluating therapies, in establishing the natural history of macular diseases, and in assessing drug toxicity.

Clinicopathologic correlations are needed on postmortem ocular tissues from patients with well-documented macular disease, particularly from the early stages of the disease. Coordinated studies utilizing biochemical, tissue culture, and other advanced techniques should be conducted.

Because of the possibility that the treatment will result in permanent visual acuity loss, argon laser photocoagulation cannot be considered for therapy in patients with aging-related maculopathy accompanied by neovascular membranes within 200 μm of the foveal avascular zone. Other wavelengths of light, produced by the krypton laser for example,[28] may offer potential advantages in this regard and should be evaluated by means of randomized controlled clinical trials.

RECOMMENDATIONS

Based on the foregoing assessment of recent accomplishments, current activities, and research needs and opportunities in "Macular Degeneration," the Panel has made the following recommendations concerning research in this subprogram over the next five years. These have been grouped under two headings: Program Base and Program Development Priorities.

The Program Base includes areas of ongoing research where the current level of activity is considered adequate, or areas of ongoing research in which there may be great need for additional activity, but where, in the Panel's judgment, little or no opportunity (new methods or insights) exists at present to justify a significant expansion of effort. Nonetheless, additional applications for research grants in these areas may be funded if they are innovative and of very high quality as determined by the NIH peer review system.

Program Development Priorities include areas of ongoing research in which new knowledge and techniques offer particular opportunities for scientific progress, or promising new areas of research in which there is little or no support at present but where there is both great need and high potential for success. Such areas are judged to warrant significantly increased support over the next five years, provided that high quality applications for research grants in these areas are forthcoming.

Program Base

- Continue follow-up of patients in the Macular Photocoagulation Study and conduct other randomized controlled clinical trials to evaluate treatments for macular disease.
- Develop noninvasive techniques to improve the detection of early defects in macular function, to evaluate therapies, to document the natural history of macular disease, and to assess drug toxicity (see Chapters 5, 7, and 13).

Program Development Priorities

- Expand biochemical and anatomical studies of Bruch's membrane, the retinal pigment epithelium, the retina, and the choroid to identify macular changes, particularly aging-related, which may contribute to subretinal neovascularization and other macular disease processes (see Chapter 8).
- Expand studies of the role of the barrier and transport properties of the retinal pigment epithelium and the retinal capillary endothelium in cystoid macular edema, central serous choroidopathy, and other macular diseases (see Chapter 8).
- Initiate epidemiologic studies of macular diseases to identify possible causative, protective, or aggravating factors.
- Search for and characterize extracellular factors that may initiate or modulate subretinal neovascularization.
- Develop experimental animal models of macular disease for research on pathogenesis and for treatment evaluation.

RESOURCE REQUIREMENTS

After reviewing current research grant support in each of these categories and assessing the need and potential for future development, the Panel has estimated the number of projects it believes are needed to carry out its recommendations in FY 1983. These estimates are shown in the table on the following page. For a discussion of the general basis and significance of these projections, see the "Summary" at the beginning of this report.

RESOURCE TABLE

MACULAR DEGENERATION

	No. of Grants FY 1981	Panel Recommendation FY 83	
		Add. Grants	Total Grants
Program Base			
A. Continue the Macular Photocoagulation Study and conduct other clinical trials to evaluate treatments for macular disease.	13*	0	13
B. Develop noninvasive techniques to improve detection of early defects in macular function, evaluate therapies, document natural history, assess drug toxicity.	2	1	3
Program Development Priorities			
A. Expand biochemical and anatomic studies of Bruch's membrane, the pigment epithelium and choroid to identify changes in macular diseases.	0	5	5
B. Expand studies on the barrier and transport properties of the RPE and capillary endothelium in macular diseases.	2	2	4
C. Initiate epidemiologic studies of macular diseases to identify possible causative, protective, and aggravating factors.	0	4	4
D. Search for and characterize extracellular factors that may initiate or modulate subretinal neovascularization.	0	2	2
E. Develop experimental animal models of macular disease.	1	2	3
Subtotal Grants	**18**	**16**	**34**
(% of Program)	**(5)**	**(14)**	**(7)**
Total Estimated Cost	**$1,418,000**	**$2,152,000**	**$3,570,000**

*Includes 13 grants for the multicenter Macular Photocoagulation Study.

REFERENCES

1. Westat, Inc: *Summary and Critique of Available Data on the Prevalence and Economic and Social Costs of Visual Disorders and Disabilities*. Rockville, MD, 1976.
2. Argon laser photocoagulation for senile macular degeneration. *Arch Ophthalmol* 100:912–918, 1982.
3. Peyman G, Spitznas M, Straatsma B: Peroxidase diffusion in the normal and photocoagulated retina. *Invest Ophthalmol Vis Sci* 10:181–189, 1971.
4. Norton A, Pihlaja D, Miller S: Experimental disciform serous retinal detachment: II. Autoradiography of photoreceptor protein metabolism. *Arch Ophthalmol* 91:474–480, 1974.
5. Ryan S: The development of an experimental model of subretinal neovascularization in disciform macular degeneration. *Trans Am Ophthalmol Soc* 77:707–745, 1979.
6. El-Mofty A, Gouras P, Eisner G, et al: Macular degeneration in rhesus monkey (*Macaca mulatta*). *Exp Eye Res* 27:499–502, 1978.
7. Eagle R, Lucier A, Bernardino V Jr, et al: Retinal pigment epithelial abnormalities in fundus flavimaculatus: A light and electron microscopic study. *Ophthalmology* 87:1189–1200, 1980.
8. Neville H, Armstrong D, Wilson B, et al: Studies on the retina and the pigment epithelium in hereditary canine ceroid lipofuscinosis: III. Morphologic abnormalities in retinal neurons and retinal pigmented epithelial cells. *Invest Ophthalmol Vis Sci* 19:75–86, 1980.
9. Aguirre G, Laties A: Pigment epithelial dystrophy in the dog. *Exp Eye Res* 23:247–256, 1976.
10. Folkman J: Tumor angiogenesis factor. *Cancer Res* 34:2109–2113, 1973.
11. Glaser B, D'Amore P, Michels R, et al: Demonstration of angiogenic activity from ocular tissues. *Ophthalmology* 87:440–446, 1980.
12. Federman J, Brown G, Felberg N, et al: Experimental ocular angiogenesis. *Am J Ophthalmol* 89:231–237, 1980.
13. Sorsky A: *Report on Public Health and Medical Subjects*. London, Her Majesty's Stationery Office, 1966, No 114.
14. Kahn H, Moorhead H: *Statistics on Blindness in the Model Reporting Area, 1969–1970. US DHEW Publ No NIH* 73:427, Washington, DC, 1973.
15. MacDonald AE: Causes of blindness in Canada. *Canad Med Ass J* 92:264–279, 1965.
16. Hyman LG: *Senile Macular Degeneration: An Epidemiologic Case Control Study*, thesis. The Johns Hopkins University, Baltimore, 1981.
17. Delaney W, Oates R: Senile macular degeneration: A preliminary study. *Ann Ophthalmol* 14:21–24, 1982.
18. Maltman BA, Mulvihill MN, Greenbaum A: Senile macular degeneration and risk factors: A case control study. *Ann Ophthalmol* 11:1197–1201, 1979.
19. Leibowitz H, et al: The Framingham Eye Study Monograph. *Surv Ophthalmol* 24(suppl):335–610, 1980.
20. Kini MM, Leibowitz HM, Colton T, et al: Prevalence of senile cataract, diabetic retinopathy, senile macular degeneration, and open-angle glaucoma in the Framingham Eye Study. *Am J Ophthalmol* 85:28–34, 1978.
21. Ganley J, Roberts J: Eye conditions and related need for medical care among persons 1–74 years, United States. *National Center for Health Statistics*, Series 11, No 228, US DHHS Publ No (NCH) 82–1678, Washington, DC, 1971–1972.
22. Sperduto R, Siegel D: Senile lens and macular changes in a population based sample. *Am J Ophthalmol* 90:86–91, 1980.
23. Klein B, Klein R: Cataracts and macular degeneration in older Americans. *Arch Ophthalmol* 100:571–573, 1982.
24. Ferris FL: Senile macular degeneration: Review of epidemiologic features. *Am J Epidemiol*, in press.
25. Vainisi SJ, Fishman GA, Wolf ED, et al: Cone-rod dystrophy in the Guinea baboon. *Trans Am Acad Ophthalmol Otolaryngol* 81(OP):725–730, 1976.
26. Godfrey W, Cross D, et al: HLA-B7 in presumed ocular histoplasmosis maculopathy. *Transplant Proc* 11:1874–1876, 1979.
27. Duquesnoy RJ, Amen K, Meredith TA: Association of presumed ocular histoplasmosis with HLA-B7 and DRW-2. *Transplant Proc* 11:1877–1878, 1979.
28. Bird AL, Grey RHB: Photocoagulation of disciform macular lesions with krypton laser. *Br J Ophthalmol* 63:669–673, 1979.

6

RETINAL DETACHMENT AND VITREOUS DISORDERS

INTRODUCTION

SEPARATION OF THE neural retina from the underlying pigment epithelium constitutes a retinal detachment. Detachments usually occur because of discontinuities (breaks) in the neural retina which may appear in a variety of forms, including retinal tears, atrophic retinal holes, giant retinal breaks extending for more than 90 degrees, and operculated retinal holes where a plug of retina has been pulled free to float in the vitreous body.

According to 1970–1974 data from a national survey, in the United States more than 25,000 first visits to physicians were made because of retinal detachment. Although a detached retina can be reattached in many instances and varying amounts of vision restored, approximately 6,000 eyes a year suffer irreparable detachment and loss of vision. To make matters worse, when a nontraumatic retinal detachment occurs in one eye, a retinal break develops in the fellow eye in a large percentage of cases.[1,2] The result is a great loss in productivity and earnings.

Retinal detachment may result from a variety of conditions. It occurs following 1 of every 50 cataract operations.[3] It can develop as a result of blood vessel disease or biochemical disorders and therefore is related to other major eye and systemic problems, including high myopia, diabetes, ocular trauma, and a variety of hereditary and congenital conditions. Many cases of blindness in patients with diabetic retinopathy, for example, ultimately result from abnormal cellular proliferation, contraction, and traction detachment of the macula.[4]

The vitreous body, a transparent gel that fills the posterior portion of the eye, plays an important role in the development of retinal detachment. It occupies approximately 80 percent of the volume of the eye and normally acts as an internal support to the eyeball. Three factors may cause a retinal detachment: fluid accumulation under the retina (Figure 1); abnormal traction on the retina in the direction of the vitreous cavity (Figure 2); and tears or holes in the retina which permit fluid vitreous to flow into the subretinal space, thereby floating the retina off the pigment epithelium (Figure 3). The most frequent type of detachment is one where retinal breaks coexist with a variable amount of vitreous traction.

The vitreous gel may lose its transparency as a result of hemorrhage from blood vessels damaged by injury or diseased by diabetes and other vascular disorders. Vitreous hemorrhage not only causes impairment of vision, it may stimulate inflammation, cellular proliferation, and the formation of vitreous membranes which may contract and detach the retina.

Substantial progress has been made in the ability to diagnose and treat vitreoretinal disorders, but ignorance of many basic cellular and molecular mechanisms stands in the way of major breakthroughs in prevention and treatment. The fundamental changes which cause the vitreous to become abnormal are unknown, as are the early biochemical and physiologic changes which cause retinal detachment.

Because the vitreous is transparent and the retina is readily observable by optical means, these structures present unparalleled opportunities for research on a number of ocular and systemic disorders. Membrane formation, blood vessel changes, and tumor growth can be easily studied and observed through the clear cornea, lens, and vitreous body. For example, research to improve examination techniques in retinal detachment is relevant to the

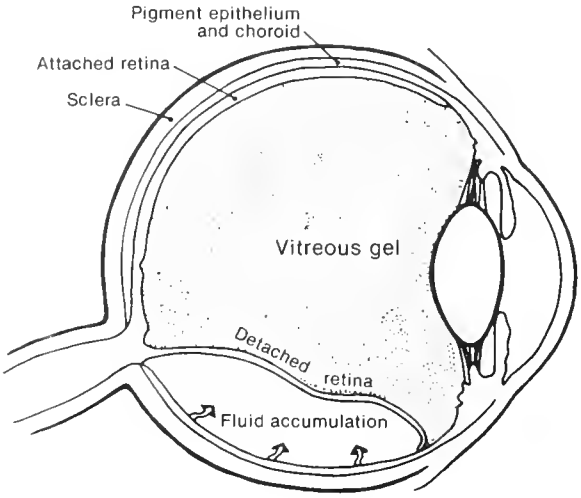


FIGURE 1. Fluid accumulation under the retina.

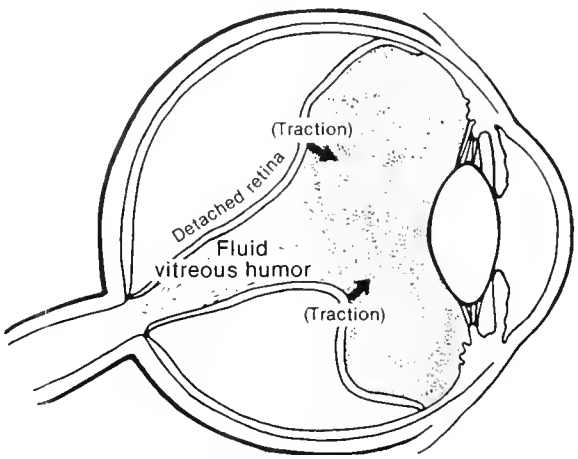


FIGURE 2. Abnormal traction on the retina.

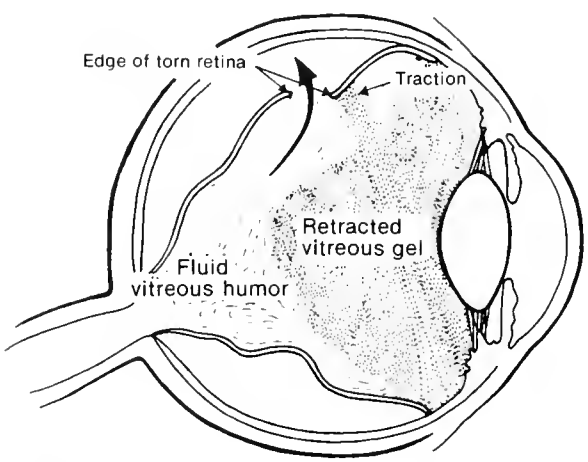


FIGURE 3. Torn retina.

diagnosis and study of virtually all diseases of the retina, choroid, and vitreous. Investigation of the retina-blood vessel interface is also important to the study of vascular and circulatory abnormalities that occur in other parts of the body where they cannot be observed directly or as easily. Observations of cellular proliferation and membrane formation in the vitreous body and the effects of various agents on them may be applicable to abnormal cellular proliferation elsewhere in the body.

SUBPROGRAM OBJECTIVES

- To understand better the development, structure, metabolism, immunologic properties, and function of the normal vitreous body and the changes that occur in aging, trauma, and disease.
- To determine the causes of abnormal cellular proliferation in the vitreous body and on the surface of the retina, membrane contraction, and retinal detachment.
- To understand the mechanisms by which the retina remains adhered to the pigment epithelium and the factors that contribute to retinal detachment and reattachment (see Chapter 8, "Retinal Pigment Epithelium").
- To find ways of preventing retinal detachments and to develop better methods for diagnosing and treating vitreoretinal disorders.

OVERVIEW OF CURRENT RESEARCH SUPPORT

In FY 1981, the National Eye Institute supported research on the vitreous body and retinal detachment through 18 research grants at a total cost of $1,722,000. More than half of these grants supported basic research on the development, structure, and function of the vitreous, the junction between the retina and vitreous, and the physiologic adhesion that normally maintains retinal attachment. Of the clinically oriented grants, some dealt with improvements in instrumentation and techniques of vitrectomy and retinal detachment surgery, others with the effects and management of trauma to the vitreous. Only a few grants supported experimental studies on cellular proliferation and traction retinal detachment.

A study of aging-related changes in the vitreous is being conducted under the auspices of the National Institute on Aging. Other research in this field is supported by the Department of Defense and the Veterans Administration. Some vitreous research is also supported by grants from Fight for Sight, Inc.

RECENT ACCOMPLISHMENTS

National Eye Institute support has led to some striking improvements in the surgical management of retinal detachment and vitreous hemorrhage. Removal of vitreous hemorrhage by vitrectomy results in improved vision in more than 60 percent of eyes, and thus has been one of the most dramatic and major breakthroughs in ocular surgery in the past decade.[5]

Significant progress has been made in restoring vision in patients affected with retinal detachment. Success rates for the usual type of retinal detachment surgery have improved from less than 40 percent in 1947 to more than 85 percent today.[6] In the 1940s, the average hospital stay for retinal detachment was four weeks, requiring much nursing care because the patient spent most of the time in bed with both eyes patched and his or her head immobilized between sandbags. As a result of improved surgical techniques, patients are now allowed out of bed the first postoperative day, and the hospital stay is reduced to six days, so that hospitalization costs have been greatly reduced.

RESEARCH NEEDS AND OPPORTUNITIES

Eighty-two leading vitreoretinal surgeons were recently asked to name the most pressing problem in their field. Seventy-six cited the dreaded complications of proliferative vitreoretinopathy. Little direct research on this problem has been supported by the NEI; thus research in this area should be emphasized if the most visually disabling vitreoretinal disorders are ever to be managed better or prevented.

Vitreous Body

With age and in degenerative conditions such as severe myopia, the vitreous gel shrinks and becomes partially liquefied. Biochemical studies on the normal gel state should be continued because they provide a basis for studies of the changes associated with gel shrinkage, liquefaction, and collapse, which leads to posterior vitreous detachment. Fundamental knowledge of posterior vitreous detachment is important because it is an underlying mechanism in the development of retinal breaks, which cause retinal detachment. In addition, posterior vitreous detachment is a very important factor in the pathogenesis of diabetic retinopathy. The detached vitreous provides a scaffold for abnormal blood vessels to grow into the vitreous cavity and later cause vitreous hemorrhage and/or traction detachment of the macula.[7] Experimental studies of posterior vitreous detachment must be conducted to gain insight into the pathogenesis of this condition which has great implications for diabetic retinopathy, rhegmatogenous detachment, and traumatic retinal detachment. Diabetic patients who develop spontaneous, total posterior vitreous separation do not develop proliferative retinopathy, and vitreous surgeons have argued that vitrectomy fundamentally alters the course of the retinopathy by mechanically inducing vitreous separation. These important observations need to be pursued experimentally.

Regeneration of hyaluronic acid, a major macromolecular component of the vitreous gel, has been demonstrated in the liquid vitreous humor of the owl monkey.[8] Very little is known, however, about this process or the extent to which vitreous gel regenerates in humans. Disorders of the regenerative process may be the underlying cause of some types of severe vitreoretinal pathology. These problems should be pursued with gel and liquid vitreous models in vivo and in vitro using vitreous cell tissue culture techniques.

Inasmuch as the vitreous body occupies 80 percent of the volume of the eye and therefore interfaces with most of its internal structures, studies of the permeability and interaction of the vitreous body with the optic nerve, lens, retina, and ciliary body are very important. The exact way in which cells and molecules from the blood gain entrance into the vitreous gel is virtually unknown. This passage of cells and molecules back and forth between the vitreous and the retina warrants extensive study with reference to the metabolism, regeneration, and immunological reaction of the vitreous body, and, most importantly, in the delivery of drugs into the vitreous gel.

The role of the vitreous body in ocular inflammatory processes is poorly understood. Vitreous cells could play an important role in the immunological response of the vitreous body, and contribute to the persistence of uveitis. Additional research in this area is urgently needed.

The most feared complication of any intraocular operation or penetrating injury is endophthalmitis, an infection involving the vitreous body and other internal structures of the eye (see Chapter 2,

"Inflammatory Disorders"). Until recently, most eyes that developed endophthalmitis were lost. Preliminary studies indicate that the most effective treatment is the injection of antibiotics directly into the vitreous cavity and the removal of the abscessed vitreous tissue by vitrectomy.[9] However, the risks of this procedure in an infected eye and the optimal dosage of intravitreous antibiotics are not fully established. Furthermore, antibiotics injected into the vitreous body may diffuse directly into the neighboring retina. Because they have profound chemical and metabolic effects, antibiotics can in certain instances poison the retina. Thus, it is important to determine the proper dosages and to test by both functional and structural analyses the retinal toxicity of different antibiotics injected into the vitreous body of animals (see Chapter 2 and Chapter 7, "Toxic and Environmental Disorders").

It is likewise important to test the efficacy of particular antibiotics, alone or in combination, in combating specific types of vitreous infections. In addition, the effect of performing vitrectomy before beginning drug therapy for endophthalmitis should continue to be compared with drug therapy alone.

An additional problem to be investigated further is the risk of sympathetic ophthalmia following vitrectomy in such cases.

Abnormal blood vessel formation within the vitreous body, which occurs in diabetic retinopathy, sickle cell disease, and occlusion of retinal veins often leads to severe vitreous hemorrhage, which itself results in loss of vision and may lead to further retinal detachment (see Chapter 1, "Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities"). Abnormal blood vessel formation on the iris (rubeosis) can cause intractable, painful, and blinding glaucoma. Rubeosis continues to be a major unsolved complication of diabetic retinopathy and is the most serious complication of vitreous surgery performed on diabetics.[10] Once the factors causing abnormal blood vessel formation have been established, more fruitful research can be conducted to prevent and treat this condition. Biochemical and biopsy studies of intraocular fluids should be pursued to determine their ability to stimulate abnormal blood vessel formation in the vitreous and on the iris.[11] Equally important are studies to ascertain the role of the vitreous and intraocular fluids in inhibiting abnormal blood vessel formation within the eye (see Chapter 1 and Chapter 5, "Macular Degeneration").

Because vitreous hemorrhage is very common in diabetes, sickle cell disease, and penetrating ocular injuries, research in this area is important. Pathological and biochemical studies should be continued to determine the role of vitreous hemorrhage in stimulating inflammation and cellular proliferation, and in causing membrane formation, contraction, and then detachment of the retina and ciliary body, resulting in functional loss of the eye.

Inasmuch as vitrectomy is of value in treating vitreous hemorrhage, its efficacy in other conditions, such as corneal and macular edema following cataract extraction, low-grade and acute inflammation, and penetrating injuries, needs to be studied. Meaningful clinical investigation of such applications must involve a sufficient number of patients to assure significant results. Randomized controlled clinical trials offer the optimal approach. Because of the relative paucity of cases in a single center and the variations in cases (for example, in eye injuries) it may be necessary to pool cases from several centers. Thus, a feasible alternative may be to conduct a multicenter collaborative trial with emphasis on strict research protocols, precise statement and measurement of clinical conditions, and advanced methods of data collection and statistical analysis.

Retinal Detachment

Major advances in the prevention and management of retinal detachment require additional basic research on the nature and strength of the normal attachment of the retina, the interface between the vitreous and retina, and studies of the blood-retina barrier (see Chapter 8 and Chapter 9, "Photoreceptors, Visual Pigments, and Phototransduction").

A number of factors are suspected of contributing to the adhesive forces which hold the retina in place, including actin filaments,[12] glycoconjugates,[13] and fluid transport. Additional research is necesssary to understand better the structural and metabolic relationships between the retina and the retinal pigment epithelium.

Proliferative vitreoretinopathy continues to be the most common cause of failure of retinal detachment surgery.[14] Membranes growing on the surface and beneath the retina will cause a retina that has been successfully reattached by surgery to redetach post-operatively. Moreover, less than one-fourth of the severe cases can be successfully treated with current surgical techniques. Detailed anatomical, biochemical, and physiological information is needed about the vitreoretinal interface if the causes of abnormal vitreous adhesion and shrinkage are to be understood. Research is desperately needed to determine what causes the formation of periretinal membranes and to find means of preventing or controlling this growth. The search for pharmacologic agents to repress these rapid cellular proliferations should be intensified and expanded.

Abnormal membranes have largely been treated by mechanical cutting and removal. Vitreous surgery instrumentation and microsurgical techniques have been developed to a highly sophisticated level,

making it possible to remove or peel abnormal membranes from large areas but not from the entire surface of the retina.[15] Although much progress has been made in the development of instruments for cutting and removing diseased or opaque vitreous, there is room for improvement. Membranes that develop following eye injuries can be tough and difficult to cut, and thin membranes in diabetic retinopathy may be attached to very weakened detached retinas. Instruments that fail to cut these membranes may cause serious tears in the retina.[16] Safer, more efficient methods of instrumentation to cut or vaporize these membranes are therefore needed.[17] In addition, new methods and improved instrumentation to photocoagulate or cauterize blood vessels in the vitreous and on the iris should be developed.

Retinal breaks currently are sealed by means of an adhesion produced with heat (diathermy), freezing (cryotherapy), or light energy (photocoagulation). However, along with their sealing effects, these techniques damage associated ocular structures to varying degrees and have varying amounts of adhesive power.[18] Further experimental studies are necessary to compare the undesirable side effects and the binding strength of the chorioretinal adhesions produced by diathermy, cryotherapy, and photocoagulation. Recent research suggests that some retinal breaks can be sealed using scleral buckling alone,[19] and further research is needed to explore these possibilities. If the need for cryotherapy, diathermy, or photocoagulation could be eliminated, so would their undesirable side effects.

Injection of air, gases, or gels such as hyaluronic acid, into the vitreous cavity to push a detached retina into place has proved of value in the management of severe retinal detachments.[20] Further studies are necessary to find better and longer lasting gases and more permanent gels, which will provide a more durable splinting of the retina against the choroid during the healing period following retinal detachment surgery.

Improved diagnostic and surgical techniques for managing retinal detachment have improved the overall success rates from 40 to 85 percent in the past 30 years. However, the success rate is not so high in complex cases such as giant retinal breaks, diabetic retinal detachments, or in retinal detachment associated with hereditary or congenital defects, severe chorioretinal degeneration, or high myopia. The factors responsible for a poor prognosis for these detachments must be isolated and investigated. The need is twofold because, unfortunately, these severe detachments have a great tendency to develop in the fellow eye.

Further improvement in results requires an increase in basic knowledge of these retinal detachments through biochemical, anatomical, and pathologic investigations. Detailed clinical investigations of the natural history of the involved and fellow eye will provide insight into pathogenesis and therefore aid in the early detection and ultimate prevention of these disabling detachments. In addition, improved understanding of natural history is basic to the evaluation of new treatments.

Giant retinal breaks continue to be a major challenge in the field of retinal detachment. The roles of various techniques including vitreous implants and injection, suturing of the retina, pars plana vitrectomy and open-sky vitrectomy, where the cornea is temporarily removed, need further evaluation.[21]

Optical instrumentation is essential for diagnosing and treating retinal detachments and vitreoretinal disorders. Continued research on optical and other imaging methods is needed to enable the examination of the vitreous body and retina in much greater detail.[22] Wide-angle ophthalmoscopy and photography, infrared and monochromatic methods,[23] holography, and specular microscopy remain among the more promising noninvasive techniques.

Not all retinal detachments are caused by holes or tears in the retina. In most transudative or exudative retinal detachments, the origin of fluid that accumulates under the retina causing it to detach remains unknown. Research on the blood-retinal barrier and the composition and transport of this fluid may give new insights into the pathogenesis and treatment of these kinds of detachments.

Retinal and choroidal circulatory deficiency or disturbances in the blood-retina barrier may be a common denominator in many patients with retinal detachment. Therefore, research must be initiated to determine the relationship between intraocular blood circulation and retinal detachment. Methods to measure regional circulatory defects in the living eye should be developed further and used in a prospective study of eyes that are thought to be at risk for the development of retinal detachment. Since more than 15 million persons are estimated to have retinal breaks without detachment, prospective studies are needed on the pathogenesis of retinal detachment.

Potential benefits to be realized from research on prevention include:

- Better selection of cases for prophylactic treatment by surgery.
- Better knowledge of predisposing factors.
- Improved knowledge of the influence of circulatory deficiencies on retinal detachment.

There continues to be a great need for animal models in retinal detachment research. Animals known to be predisposed to retinal detachment, such as those with an elongated eyeball found in myopia, should be acquired and bred. A model of

vitreoretinal membrane shrinkage, produced by noninvasive manipulations, must be developed. Equally important are studies of the isolated mammalian eye perfused with whole blood for the study of circulatory changes in the retina and choroid. Research on the influence of drugs on those tissues, and microscopic observations of changes brought about by chemical or traumatic injuries or diseases of the choroid and retina must be pursued.

RECOMMENDATIONS

Based on the foregoing assessment of recent accomplishments, current activities, and research needs and opportunities in "Retinal Detachment and Vitreous Disorders," the Panel has made the following recommendations concerning research in this subprogram over the next five years. These have been grouped under two headings: Program Base and Program Development Priorities.

The Program Base includes areas of ongoing research where the current level of activity is considered adequate, or areas of ongoing research in which there may be great need for additional activity, but where, in the Panel's judgment, little or no opportunity (new methods or insights) exists at present to justify a significant expansion of effort. Nonetheless, additional applications for research grants in these areas may be funded if they are innovative and of very high quality as determined by the NIH peer review system.

Program Development Priorities include areas of ongoing research in which new knowledge and techniques offer particular opportunities for scientific progress, or promising new areas of research in which there is little or no support at present but where there is both great need and high potential for success. Such areas are judged to warrant significantly increased support over the next five years, provided that high quality applications for research grants in these areas are forthcoming.

Program Base

- Conduct research on the development, structure, metabolism, and immunological properties of the vitreous body and its cellular elements and the changes that occur in aging, trauma, and disease.
- Conduct detailed anatomical, biochemical, and physiological studies on the vitreoretinal interface; studies of the precise way in which cells and molecules from the circulatory system gain entrance to the vitreous body; and, studies of the permeability and metabolic interactions of the vitreous body with adjacent tissues, particularly the ciliary body and lens.

Program Development Priorities

- Expand research on finding the cause of and preventing abnormal cellular proliferation, membrane formation, contraction, and retinal detachment; on determining the roles of posterior vitreous detachment and vitreous hemorrhage in retinal detachment; and on developing animal models for research in this area.
- Determine the mechanisms by which the retina remains adhered to the retinal pigment epithelium, the factors that may contribute to detachment, the factors which may maintain and enhance the viability of the detached retina, and the relationship between disturbances in the blood-retinal barrier and retinal detachment (see Chapter 8).
- Improve instrumentation and continue to develop and evaluate new methods for the diagnosis and treatment of vitreoretinal disorders (see Chapter 13, "Noninvasive Techniques in the Study of Retinal Disorders").

RESOURCE REQUIREMENTS

After reviewing current research grant support in each of these categories and assessing the need and potential for future development, the Panel has estimated the number of projects it believes are needed to carry out its recommendations in FY 1983. These estimates are shown in the table on the following page. For a discussion of the general basis and significance of these projections, see the "Summary" at the beginning of this report.

RESOURCE TABLE

RETINAL DETACHMENT AND VITREOUS DISORDERS

	No. of Grants FY 1981	Panel Recommendation FY 83	
		Add. Grants	Total Grants
Program Base			
A. Conduct research on properties of the vitreous body and changes in aging, trauma, and disease.	9	−2	7
B. Conduct studies on the vitreoretinal interface.	1	1	2
Program Development Priorities			
A. Expand research on abnormal cellular proliferation; posterior vitreous detachment and hemorrhage in retinal detachment; developing animal models.	3	6	9
B. Determine mechanisms for retinal adherence to retinal pigment epithelium and factors to enhance the viability of the detached retina.	2	2	4
C. Improve instrumentation and new methods for diagnosis and treatment of vitreoretinal disorders.	3	2	5
Subtotal Grants	**18**	**9**	**27**
(% of Program)	**(5)**	**(8)**	**(5)**
Total Estimated Cost	**$1,722,000**	**$1,113,000**	**$2,835,000**

REFERENCES

1. Ashrafzadeh MT: I. Preoperative findings. *Arch Ophthalmol* 89:476–483, 1973.
2. Freeman HM: Fellow eyes of giant retinal breaks. *Trans Am Ophthalmol Soc* 76:343–382, 1978.
3. Norton EWD: Retinal detachment in aphakia. *Am J Ophthalmol* 58:111–124, 1964.
4. Davis MD: Vitreous contraction in proliferative diabetic retinopathy. *Arch Ophthalmol* 74:741–751, 1965.
5. Blankenship G: Pars plana vitrectomy for diabetic retinopathy: A report of eight year's experience. *Mod Probl Ophthalmol* 20:376, 1979.
6. Hilton GF, McLean EB, Norton EWD: Retinal detachment. *American Academy of Ophthalmology Manual*, Rochester, Minnesota, 1979.
7. Michels RG: Surgical objectives and techniques, in Michels RG (ed): *Vitreous Surgery*. St Louis, CV Mosby Co, 1981, pp 135–207.
8. Osterlin SE, Jacobson B: The synthesis of hyaluronic acid in vitreous: III. In vivo metabolism in the owl monkey. *Exp Eye Res* 7:524, 1968.
9. Diamond JG: Intravitreal management of endophthalmitis, in Shimizu K, Oosterhuis JA (eds): *Acta XXIII Concilium Ophthalmologicum*. Amsterdam, Excerpta Medica, 1979, p 1571.
10. Michels RG: Vitrectomy for complications of diabetic retinopathy. *Arch Ophthalmol* 91:237, 1978.
11. Glaser BM, D'Amore PA, Michels RG, et al: Identification of vasoproliferative activity from mammalian retina. *J Cell Biol* 84:298, 1980.
12. Burnside B, Laties AM: Actin filaments in apical projection of the primate pigmented epithelial cell. *Invest Ophthalmol Vis Sci* 15:570–575, 1976.
13. Adler HA, Severin KM: Proteins of the bovine interphotoreceptor matrix: Tissues of origin. *Exp Eye Res* 32:755–769, 1981.
14. Machemer R, Van Horn DL, Aaberg TM: Pigment epithelial proliferation in human retinal detachment with massive periretinal proliferation. *Am J Ophthalmol* 84:383, 1977.
15. Machemer R, Laqua H: A logical approach to the treatment of massive periretinal proliferation. *Ophthalmology* 85:554, 1978.
16. Michels RC: Postoperative management and complications, in Michels RG (ed): *Vitreous Surgery*. St Louis, CV Mosby Co, 1981, pp 369–435.
17. Miller JB, Smith MR, Boyer DS: Intraocular carbon dioxide laser photocautery. *Ophthalmology* 87:1112–1120, 1980.
18. de Guillebon H, Tribonniere MM, Pomerantzeff O: Adhesion between retina and pigment epithelium. *Arch Ophthalmol* 86:679–684, 1971.
19. Fetkenhour K, Hauch T: Scleral buckling without thermal adhesion. *Am J Ophthalmol* 89:662–666, 1980.
20. Tolentino FI, Schepens CL, Freeman HM (eds): *Vitreoretinal Disorders*. Philadelphia, WB Saunders, 1976, p 492.
21. Schepens C: Clinical and research aspects of subtotal open-sky vitrectomy. 37th Jackson Memorial Lecture. *Am J Ophthalmol* 91:143–171, 1981.
22. Pomerantzeff O, Fish HB, Schepens CL: Wide-angle optical model of the eye, in Pruett RC, Regan CDJ (eds): *Retina Congress*. New York, Appleton-Century-Crofts, 1974, pp 91–100.
23. Ducrey NM, Delori FC, Gragoudas ES: Monochromatic ophthalmoscopy and fluorescein angiography. *Arch Ophthalmol* 97:1349–1350, 1979.

7

TOXIC AND ENVIRONMENTAL DISORDERS

INTRODUCTION

THE INCIDENCE or prevalence of toxic and environmental disorders of the eye is difficult to estimate. Yet, from clinical experience and pertinent research, it is known that many drugs, chemicals, environmental factors, and pollutants can damage the eye.

For the retina, the problem of toxicity is twofold. First, in its susceptibility to toxic agents the retina differs from all other tissues of the body: it has special nutritional requirements and an intricate and delicately balanced physiology, and it functions in a rigorously controlled environment, which has a surprisingly narrow tolerance for change. Thus, a variety of agents, acting separately or at times together, can severely damage the retina, although they are apparently harmless to other tissues. For instance, drugs that do little or no harm elsewhere in the body can produce a retinal degeneration.[1] Also, the retina can be injured by exposure to levels of ambient light too weak to affect any other tissue.[2] Consequently, the only system that can predict the effect of a given drug or environmental hazard on the retina is the retina itself.

Second, current studies of toxic and environmental disorders of the retina and choroid are inadequate in scope and quality. As a general rule, they are performed not by vision researchers, but by personnel without any special knowledge of the eye. For the most part, these studies are not supported by the National Eye Institute.

At the root of many of the problems surrounding retinal toxicity is the way in which drugs are developed. In the United States, most drug testing is done by individual pharmaceutical companies or specialized commercial testing laboratories. Although it is true that the eye receives more attention today than it did in the past, ocular toxicity testing is still inadequate; pharmaceutical companies and testing laboratories usually lack the necessary in-house expertise in ocular physiology and pathology.

When drugs are developed for systemic use, the Food and Drug Administration requires that their potential toxicity to the eye be evaluated before human testing begins. However, such studies vary widely in quality and often are superficial. In fairness, it should be added that even good will and substantial scientific attention are no guarantees against the possibility of late ocular complications. Toxic effects on the eye can be subtle or slow to develop and thus may only become evident sometime after even a well-tested drug is put on the market. These effects may not be recognized for what they are, even by an eye specialist unless he or she has some special reason for suspicion. Tests such as dark adaptation, electroretinography, or electrooculography, which might indicate early toxic effects, are not routinely performed. Many instances of drug toxicity are picked up through reporting efforts of the National Registry of Drug Induced Ocular Side Effects.

The situation is better when drugs are developed directly for use in ocular therapy. As a general rule, those who undertake this work have greater familiarity with the anatomy and physiology of the eye, and they are primarily concerned with its proper function.

Environmental pollutants and poisons constitute yet another source of danger to the eye. For instance, the use of organophosphorus derivatives for pest control as documented by Ishikawa[3] led to thousands of cases of myopia accompanied by toxic neuropathy in the Saku District of Japan. Similarly, the Japanese have also experienced a widespread outbreak of optic neuritis and peripheral neuropathy

after the dumping of mercury residue into Minamata Bay. [4]

SUBPROGRAM OBJECTIVES

- To improve the scope and quality of clinical and basic research relating to toxic and environmental disorders of the eye.
- To gather laboratory and clinical information about such hazards in an efficient manner.
- To disseminate information about potential hazards at the earliest possible moment, thus limiting exposure.

OVERVIEW OF CURRENT RESEARCH SUPPORT

Toxic and environmental hazards in general are the concern of numerous branches of government and private foundations as well as widely differing segments of industry.

As a general rule, the hazards from sudden bursts of radiant energy have commanded the greatest attention and are the most thoroughly investigated. Hazards resulting from chronic exposure to low levels of drugs and chemicals have received the least attention.

In FY 1981 the National Eye Institute supported two projects at a total cost of $91,000 in which research on the toxic and environmental disorders of the eye was a primary concern. However, research on toxic ocular effects was included within projects supported under other Retinal and Choroidal Diseases subprograms in FY 1981. These studies concerned the retinal toxicity of exogenous chemicals, drugs, and various intravenously administered fluorescent dyes used for angiographic diagnosis of retinal vascular disorders. In addition, the NEI supported eight projects in other programs on the adverse effects of drugs and poisons on the cornea, lens, aqueous outflow channels, and optic nerve. Intramural studies were also under way at the National Eye Institute on possible ocular complications of anticancer drugs and the nature of drug metabolizing and detoxifying enzymes which determine the fate of drugs in the eye.

Each of the other Panel reports in this National Plan covering the National Eye Institute disease programs addresses specific problems and research needs presented by diverse toxic and environmental disorders.

RECENT ACCOMPLISHMENTS

An ever widening range of medications are entering the pharmacopoeia: drugs to control the rhythm of the heart directed at individual types of receptors, drugs to alter mental functions based on their similarity to normally occurring neurotransmitters, and drugs to control the spread of neoplasms through disruption of cellular organelles such as microtubules. In view of the ubiquity of cellular receptors and neurotransmitters, virtually all drugs administered systemically affect many organs. In some instances toxicity is dose related; in others it is idiosyncratic. Sometimes it occurs after long usage and hence can be puzzling when first seen.

Special attention needs to be paid to the potential of anticancer drugs for producing ocular toxicity. When any of the following agents are used, frequent, careful ophthalmic examination is mandatory.

- Vinca alkaloids (vincristine and vinblastine, for example) are plant alkaloids known to depolymerize microtubules. Their use is associated with a range of ophthalmic side effects, including ptosis, ophthalmoplegia, and optic neuritis. [5] The optic neuritis apparently follows damage to retinal ganglion cells. If caught in time, symptoms can be reversed by discontinuing the drug.
- Tamoxifen, a triphenylethylene anti-estrogen, has been reported to cause macular edema accompanied by small, white, intraretinal, refractile opacities. [6,7] A mild keratopathy characterized by whorl-like superficial opacities is frequently present. In one patient, widespread neurotoxicity, including hearing loss, was also evident.
- Adriamycin, daunomycin, and daunorubicin and related compounds enhance the formation of semiquinones. Blurred vision has been reported in patients undergoing chemotherapy with these drugs. [8] In the rat, it has recently been shown that adriamycin can cause a degeneration of photoreceptors.

Organic solvents such as benzene, vinyl benzene (styrene), methyl benzene (toluene), and n-hexane are widely used in industry. In virtually all industrial situations, reasonable safety standards have been established. Most industrial injuries to the eye result from a lapse in normal procedure or, on rare occasions, failure to comprehend a risk. [9] More

dangerous at present is the rapid increase of solvent inhalation as a form of drug abuse. Toxic reaction to paint sniffing, generally of toluene vapors, has become increasingly common in Mexico and the United States. Unfortunately, toluene is neurotoxic;[10] cerebellar damage is common, and optic neuritis has been reported recently as well.

Largely as the result of the work of Potts and others, the ocular toxicity of methanol poisoning is understood reasonably well, and some treatments are possible.[11] Further research on the management of ocular methanol toxicity seems warranted in view of the possible increase in its production to serve as an alternative to or extender for gasoline in motor vehicles.

The widespread industrial use of metals and worldwide dumping of metallic compounds has made it difficult to establish an association between the hazards and the pathology in an individual patient. The example of Minamata disease—sensory disturbance, impaired hearing, and cerebellar signs accompanied by visual field constriction—can illustrate this problem.[4] Minamata disease was brought about by an industrial discharge of methyl mercury and a progressive increase in toxin concentration as it passed up the food chain from plankton, to fish, to man. Further, because the disease has a late onset after ingestion of the toxin, its effects only became evident a long time after the original pollutant discharge. Had large numbers of people not been affected, a diagnosis might never have been made.

In the late 1960s and early 1970s, when confronted with an endemic pocket of optic neuritis, peripheral neuropathy, and myopic astigmatism in the Saku district of Japan, Ishikawa and Ohto began a broad search for a toxic agent.[12] Finding that in some respects the symptoms were reminiscent of those of patients who had been treated with organophosphorous eyedrops for myasthenia gravis, glaucoma, strabismus, or amblyopia, they measured the cholinesterase activity of the serum (organophosphate compounds produce an intense cholinomimetric response by reducing cholinesterase activity) and assayed plasma levels of organophosphorus derivatives. The serum cholinesterase level was found to be decreased in the blood of most individuals who were exposed, and was inversely proportional to the levels of organophosphate.

It has long been realized that organophosphorus compounds can be neurotoxic. Now it seems likely that chronic exposure to organophosphate pesticides did cause myopia in children of school age in addition to—or separate from—an optic neuropathy.[13] Chronic organophosphorus intoxication serves as an excellent example of a complex interaction between an environmental hazard and a susceptibility that is both age- and gene-related. Thanks largely to the excellent studies of Ishikawa, several key elements in this public health disaster in Japan associated with organophosphate pesticide use have been brought to light. The full implication of the findings has yet to be worked out.[3]

RESEARCH NEEDS AND OPPORTUNITIES

The great majority of toxic and environmental agents that affect vision do so by damaging the retina and optic nerve or the lens. In many instances, for example, exposure to industrial solvents, the dangers are well understood; in others, for example, exposure to drugs such as the phenothiazines, the dangers are only partially understood. In all instances, precise definition of risk depends on careful studies relating exposure to the incidence of injury. For this reason, a substantial opportunity exists to apply modern case control epidemiological techniques to these problems. Moreover, the power of these methods, if properly applied, might disclose relationships that at present can only be suspected.

In the laboratory, several major initiatives could be undertaken. For instance, in the evaluation of a drug's potential toxicity to the eye, it is very important to know the precise biodistribution of the drug. Powerful new techniques such as whole organ autoradiography have been perfected, and these techniques should be used for the ocular localization of each new drug proposed for ophthalmic use. Likewise, drug-related changes in important aspects of ocular physiology such as regional blood flow and tissue metabolism should be monitored. Moreover, advantage should be taken of recent progress in organ and tissue culture of the retina and lens for in vitro evaluation of the toxicity of new drugs prior to release for public use.

In an attempt to save vision threatened by conditions once deemed hopeless, a variety of procedures, including vitreous infusion of antibiotics for endophthalmitis and vitrectomy for intravitreal hemorrhage, have entered ophthalmic practice.[14] To insure that the benefits sought are realized, careful studies are needed to define the retinal toxicity of agents introduced into the vitreous cavity. This is especially important in the treatment of endophthalmitis in which large concentrations of antibiotics may be administered.

Infection by organisms usually considered nonvirulent are a dreaded complication of the immune suppression necessary to prepare a recipient for a tissue transplant or to treat certain forms of autoimmune disease. Immune suppression—and subsequent infection—also occurs as a side effect of some forms of cancer chemotherapy.[15] Less commonly, but no less dangerous, such infections can follow prema-

ture birth or occur in those with congenital or acquired immune system defects. Whatever the cause, the therapeutic problem is much the same. The eye is prominent among the structures at risk.

Such infection may be caused by fungi, such as Candida or Aspergillus, parasites such as *Toxoplasma gondii*, or herpes virus or cytomegalovirus (see Chapter 2, "Inflammatory Disorders" and *Volume Two, Part Three, Report of the Corneal Diseases Panel*, Chapter 1, "External Ocular Infections and Inflammatory Disease"). Several strategies have been devised to combat ocular infections under these circumstances, including special antibiotics or special treatments such as intravitreal deposition and transfer enhancement of the immune mechanism. In view of the increasing numbers of individuals likely to develop such infections, further research in these areas should be encouraged.

RECOMMENDATIONS

Based on the foregoing assessment of recent accomplishments, current activities, and research needs and opportunities in "Toxic and Environmental Disorders," the Panel has made the following recommendations concerning research in this subprogram over the next five years. These have been grouped under two headings: Program Base and Program Development Priorities.

The Program Base includes areas of ongoing research where the current level of activity is considered adequate, or areas of ongoing research in which there may be great need for additional activity, but where, in the Panel's judgment, little or no opportunity (new methods or insights) exists at present to justify a significant expansion of effort. Nonetheless, additional applications for research grants in these areas may be funded if they are innovative and of very high quality as determined by the NIH peer review system.

Program Development Priorities include areas of ongoing research in which new knowledge and techniques offer particular opportunities for scientific progress, or promising new areas of research in which there is little or no support at present but where there is both great need and high potential for success. Such areas are judged to warrant significantly increased support over the next five years, provided that high quality applications for research grants in these areas are forthcoming.

Again, it should be noted that additional recommendations pertaining to specific studies of ocular toxicity and environmental hazards may be found within the reports of the other planning panels.

Program Base

- Conduct research on toxic and environmentally induced disorders of the eye, with an emphasis on both epidemiological studies and studies which localize the cellular sites of drug action and which determine the mechanism of toxicity.

Program Development Priority

- Develop screening systems for potential ophthalmic toxicity, including in vivo physiological tests and in vitro tests of cultured ocular tissue.

RESOURCE REQUIREMENTS

After reviewing current research grant support in each of these categories and assessing the need and potential for future development, the Panel has estimated the number of projects it believes are needed to carry out its recommendations in FY 1983. These estimates are shown in the table on the following page. For a discussion of the general basis and significance of these projections, see the "Summary" at the beginning of this report.

RESOURCE TABLE

TOXIC AND ENVIRONMENTAL DISORDERS

	No. of Grants FY 1981	Panel Recommendation FY 83	
		Add. Grants	Total Grants
Program Base			
A. Conduct research on toxic and environmental disorders of the eye.	2	0	2
Program Development Priority			
A. Develop screening systems for ophthalmic toxicity.	0	2	2
Subtotal Grants	**2**	**2**	**4**
(% of Program)	**(1)**	**(2)**	**(1)**
Total Estimated Cost	**$91,000**	**$329,000**	**$420,000**

REFERENCES

1. Bernstein HN: Chloroquine ocular toxicity. *Surv Ophthalmol* 12:415–447, 1967.
2. Goldman AI, Ham WT Jr, Mueller AH: Ocular damage thresholds and mechanisms for ultrashort pulses of both visible and infrared laser radiation in the rhesus monkey. *Exp Eye Res* 24(1):45–56, 1977.
3. Ishikawa S, Miyata M: Development of myopia following chronic organophosphate pesticide intoxication: An epidemiological and experimental study, in Merigan WH, Weiss B (eds): *Neurotoxicity of the Visual System.* New York, Raven Press, 1980, pp 233–254.
4. Takeuchi T, Eto K: Pathology and pathogenesis of Minamata disease, in Tsubaki T, Irukayama K (eds): *Minamata Disease.* New York, Elsevier-North Holland, 1977, pp 103–141.
5. Norton SW, Stockman JA III: Unilateral optic neuropathy following vincristine chemotherapy. *J Pediatr Ophthalmol Strabismus* 16:190–193, 1980.
6. Kaiser-Kupfer MI, Lippman ME: Tamoxifen retinopathy. *Cancer Treat Rep* 62:315–320, 1978.
7. McKeown CA, Swartz M, Blom J, et al: Tamoxifen retinopathy. *Br J Ophthalmol* 65:177–179, 1981.
8. Kretzer F, Mehta R: An age-dependent, adriamycin-induced photoreceptor atrophy in albino rats. *Invest Ophthalmol Vis Sci* 20(suppl):40, 1979.
9. Raitta C, Seppalainen A-M, Huuskonen MS: N-hexane maculopathy in industrial workers. *Albrecht Von Graefes Arch Klin Exp Ophthalmol* 209:99–110, 1978.
10. Keane JR: Toluene optic neuropathy. *Ann Neurol* 4:390, 1978.
11. Gilger AP, Potts AR: Studies on the visual toxicity of methanol: V. The role of acidosis in experimental methanol poisoning. *Am J Ophthalmol* 39:63–86, 1955.
12. Ishikawa S, Ohto K: Optic-neuropathy by environmental exposure to organophosphate pesticides. *Proc 5th Afro-Asian Congress of Ophthalmol*, Tokyo, 1972, pp 434–444.
13. Tamura O, Mitsui Y: Organophosphorous pesticides as a cause of myopia in school children: An epidemiological study. *Jpn J Ophthalmol* 19:250–253, 1975.
14. Morgan BS, Larson B, Peyman GA, et al: Toxicity of antibiotic combinations for vitrectomy infusion fluid. *Ophthalmic Surg* 10:74–77, 1979.
15. Doft BH: Immunity, cytomegalovirus, and the eye, in Smith JL (ed): *Neuro-Ophthalmology Focus 1980.* New York, Masson Publishing USA, Inc, 1979, pp 419–425.

FUNDAMENTAL PROCESSES AND RETINAL DISORDERS

8

RETINAL PIGMENT EPITHELIUM

INTRODUCTION

THE RETINAL PIGMENT epithelium (RPE) is a specialized monolayer of cells interposed between the choroidal capillaries and the photoreceptors of the neurosensory retina. Because the RPE controls the exchange of materials between the photoreceptors and their blood supply, it is as vital to the integrity of the visual process as the photoreceptors themselves. Indeed, the two cell types usually are considered a functional unit.

The photoreceptors' dependence on the RPE stems from a number of factors. One of the first to be recognized is the role played by the RPE in the rhodopsin cycle. More than 100 years ago, Kuhne showed that the regeneration of visual pigment from its bleached photoproducts required the presence of the RPE. We know now that, with the aid of specialized transport molecules (retinol-binding proteins), the RPE takes up vitamin A from the retina and bloodstream, esterifies and stores it internally, and makes it available to the visual cells to replenish their supply of photopigment.

The RPE serves also as a barrier between the bloodstream and the photoreceptors (Figure 1). By selectively regulating the transport of metabolites to and from the visual cells, the RPE regulates their environment. By virtue of the cellular processes with which it envelops the light-sensitive portions of the photoreceptors (the outer segments), the secretion of complex molecules called mucopolysaccharides (glycosaminoglycans), and the movement of fluids in conjunction with its ion transport activities, the RPE is thought to create some of the forces that cause the neurosensory retina to remain in its proper position. The RPE absorbs excess light energy that is not trapped by the photoreceptors themselves, thereby reducing light scatter and improving the clarity of images. Finally, this remarkable layer of cells, in addition to nurturing the photoreceptors, also plays a central role in keeping them youthful and vigorous. On a daily basis, it eats and digests portions of the photoreceptor outer segments that are discarded with the rising and setting of the sun.

If any of these functions are disturbed, the photoreceptors and vision are adversely affected. Thus, it would not be surprising if the RPE were involved in a wide variety of ocular diseases, including hereditary and developmental disorders, aging-related degeneration, circulatory or inflammatory disease, retinal detachment, and light- and drug-induced retinopathy.

The basal surface of the RPE rests against a fibroelastic membrane called Bruch's membrane, which is itself interposed between the choroidal capillary bed and the RPE. Bruch's membrane is a complex layer of collagen and elastic fibers embedded in a matrix.[1] The pigment epithelium contributes to the formation of Bruch's membrane by secreting a thin collagenous layer known as the basement membrane of the RPE.[2] Bruch's membrane gives mechanical support to this region and constitutes a barrier to the abnormal growth of blood vessels (neovascularization) from the choroid into the photoreceptor region. However, during certain disease processes, this barrier is breached by sprouting vessels which eventually hemorrhage or interfere with the optical properties of the region.

In addition, pathological elevations called drusen frequently appear in Bruch's membrane as a result of aging or genetic factors. The precise mechanism for their formation is not known, although several theories have been advanced.[3–5] Drusen can cause serious defects in vision when they become sufficiently large to distort the RPE and the underlying photoreceptors. Their presence is most sorely no-

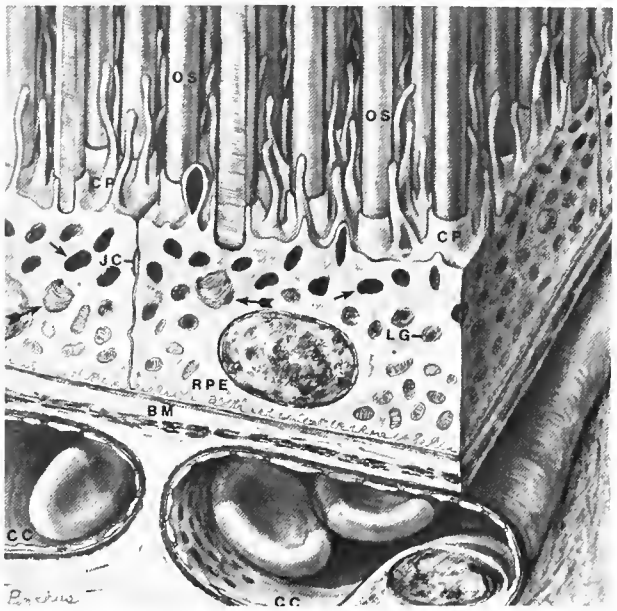


FIGURE 1. Rendition of the retinal photoreceptor-pigment epithelial complex and its adjacent blood supply. The light-sensitive outer segments (OS) of photoreceptors are in very close association with cellular processes (CP) of the retinal pigment epithelium (RPE). The RPE rests on Bruch's membrane (BM), a fibroelastic layer that separates the photoreceptor-RPE unit from its blood supply, the latter represented here by a layer of choroidal capillaries (CC). Melanin granules (small arrows) within the cytoplasm of the RPE prevent the scattering of excess light by absorbing it. Lipofuscin granules (LG), incompletely digested portions of outer segment fragments and various intracellular components, are visible as are the phagosomes (large arrows) from which many of the lipofuscin granules originate. Phagosomes are recently ingested tips of photoreceptor outer segments that are shed into the extracellular space and "eaten" by the RPE. The RPE cells are joined together at their lateral surfaces by junctional complexes (JC). Included in these complexes are tight junctions which, in concert with similar junctions in retinal capillaries, serve as the site of the blood-retinal barrier. (Adapted from Young RW: A theory of central retinal disease, Chapter 14, in Sears ML (ed): *New Directions in Ophthalmic Research*, Yale University Press, New Haven and London, 1981.)

cytoplasm is made possible by differences between the chemical composition of its basolateral plasma ticed when they disturb the RPE underlying the fovea, the point of most acute central vision.

The RPE intervenes between two tissue microenvironments, the capillary bed of the choroid and the subretinal space (the retinal ventricle). This space can enlarge considerably when the photoreceptors detach from the RPE.

In keeping with its epithelial nature, the RPE accomplishes its barrier and transport functions by two means. Portions of the lateral surfaces of adjacent RPE cells are sealed together by tight junctions that allow only a limited diffusion of substances into and out of the retina by extracellular pathways.[6,7] These tight junctions therefore dictate that most metabolites and larger molecules pass across the cellular barrier by intracellular pathways. Directional transport of substances across the RPE's membrane, the basal part of which rests on Bruch's membrane, and that of its apical plasma membrane, which faces the photoreceptors of the neurosensory retina. For example, sodium-potassium pumps are located only on the apical plasma membrane[8,9] which is studded with numerous cytoplasmic processes. Because sodium is pumped only to the extracellular side of the plasma membrane in which the pump molecules are embedded, the RPE is capable of actively directing a net flow of sodium in the direction of the photoreceptors.[10]

The cytoplasmic processes on the apical surface of the RPE surround the outer segments of both rods and cones, and are closely apposed to them. In the human retina, these processes can take on a sheet-like form and ensheath the rod and cone outer segment tips.[11,12] These processes participate in a remarkable function of the RPE: the phagocytosis of rod and cone outer segment tips that are cast off on a daily basis.[13–16] The photoreceptors shed their outer segment tips into the subretinal space, and the RPE clears up the resulting debris by a process of recognition, ingestion, and digestion known as phagocytosis.[17]

The mechanism whereby the RPE recognizes and ingests photoreceptor outer segment tips presents an intriguing and unsolved problem, which is actively being studied. Rats with inherited retinal dystrophy (rdy/rdy) exhibit a defect in this phagocytic mechanism[18–21] and serve as a useful model for studying aberrations in this important aspect of RPE function. Curiously, the rods shed the bulk of their tips after sunrise,[22–24] whereas the cones shed most of theirs after sunset.[25–27] Thus, in addition to its other chores, some of which remain to be mentioned, the RPE is faced with an enormous phagocytic burden. The cells carry out these activities on a daily basis from the time that photoreceptors first appear in utero until the death of the individual some 70 or more years later.

The RPE-photoreceptor interface is also thought to be the site at which adhesive forces hold the neurosensory retina smoothly and snugly against the choroid. A number of factors are thought to contribute to these adhesive forces, but none is known with certainty.[28–30] The combination of ensheathing RPE cytoplasmic projections that contact the photoreceptors in concert with a thin film of interphotoreceptor matrix containing mucopolysaccharides (glycosaminoglycans) interspersed between them is believed to contribute somewhat to retinal adhesion. The cellular source of these molecules has been attributed both to the photoreceptors and the RPE. Transport of fluid out of the subretinal space, thereby reducing its volume, is also thought to be a factor in adhesion.[30] The mechanisms for transporting water out of the subretinal

space and into the choroidal circulation are complex and by no means completely understood. Fluid transport across other epithelia is osmotically linked to active solute transport.[31] For example, in gall bladder epithelium, fluid transport is tightly coupled to the transport of sodium chloride.[32] Nonetheless, metabolic pumps, one of which has been carefully localized to the apical surface of the RPE,[9,33,34] no doubt also play a crucial role in the adhesion of the neurosensory retina to the RPE and choroid.

It has been recognized for many decades that the RPE, by virtue of its melanin granules, efficiently absorbs light not captured by the photoreceptors. The optical advantages of this light-trapping ability are two-fold. The absorption of "excess" light lessens glare (backscattering of photons), thereby improving resolution of the visual image. Also, the RPE pigment acts as an adjunct to the melanin in the choroid by blocking the wrong-way transmission of light through the sclera.

Another type of pigment found in the RPE is the lipofuscin granules[35] or residual bodies. These are the indigestible products of the lifelong history of phagocytosis and autophagic (self-eating) activity carried out by this cell layer.[12,13,36] Phagocytosis of outer segment tips was discussed earlier in this chapter. Autophagy is a process carried out by most cells in the body, including the RPE,[37] whereby surplus or "worn-out" intracellular organelles such as mitochondria and cytoplasmic membranes are destroyed.

Although there is no evidence that the RPE can itself respond to normal levels of environmental light, it does produce a fast photovoltage believed to be thermoelectric in response to intense flash stimuli[38] and generates slow electrical responses to the ionic and chemical events that follow the reception of light by photoreceptors. These latter responses are not present in the clinical electroretinogram (ERG), which includes only the short duration (milliseconds) a and b wave components. Slow electrical potentials produced at least in part by the RPE appear on the order of seconds and minutes following photostimulation. Stable recordings of these responses are more difficult to make than those observed in the clinical ERG and special techniques must be used involving direct current amplification and avoidance of ocular movements.

The first of two electrical peaks with a slow time course is the c-wave,[39–41] a response that appears several seconds after the onset of light. The cellular origin of the c-wave is known to be RPE-dominated, and the ionic mechanisms responsible for its generation are reasonably well understood.[42,43] However, many problems must be resolved before this recording can be used as a clinical test for RPE function.

The c-wave of the ERG is followed by a change in the standing potential that appears some minutes later.[44,45] Mechanisms underlying the standing potential are complex, involving cellular elements and ionic events that have not been fully defined. However, it appears that the light rise in the standing potential, which forms the basis for a test called the electro-oculogram (EOG),[46] is generated across the basal membrane of the RPE.[47,48]

In view of the diverse roles of the RPE in maintaining retinal integrity and function, one would expect to find numerous disorders that originate in the RPE itself. However, due to the close functional interrelationships between the RPE and the photoreceptors, it is difficult even under the ideal conditions of animal model studies to pinpoint unequivocally the primary cell type in which a given disease is expressed.

The dystrophic rat retina is an example of an animal model for inherited retinal disease in which most investigators agree that the RPE represents the primary site of mutant gene expression. This agreement derives from an elegant experiment in which the cells of normal and mutant animals were mixed shortly after conception and the embryo thereafter allowed to grow to term in a host female.[21] Following growth and postnatal maturation, the retina was examined histologically. It was observed that degeneration of photoreceptors occurred only when they were in contact with RPE derived from mutant parents. Photoreceptors contacting the cells of normal parents did not degenerate. Classic efforts of this kind are only possible in small animals such as rodents with high fertility and maturation rates. The case for mutant gene expression in the RPE of other animal models remains open.

It has been suggested that central progressive retinal atrophy in dogs is a pigment epithelial dystrophy because these cells appear to be affected first.[49,50] However, the genetics of this disorder are not well understood.[51]

In the human, the problem of determining whether a primary disease process rests within the RPE is formidable. No one can say with certainty whether there are any primary dystrophies of the human RPE, although it is likely that they exist. What is lacking is an ability to correlate evidence gathered by noninvasive clinical techniques with biochemical and morphological data. The latter are virtually nonexistent due to a lack of human material that has been examined in the early stages of degeneration.

Studies of the RPE are relevant to numerous areas of retinal research and disease because of its crucial role in retinal function. Detachments of the RPE can be part of a syndrome known as central serous choroidopathy, a condition in which fluid accumulates between the photoreceptors and the RPE. Whether the detachment and observed "leakiness" of the RPE is the primary defect in this condition is an unresolved controversy. In fact, the

etiology of RPE detachment is unknown. Involvement, direct or indirect, of the RPE has been inferred in many retinal and choroidal diseases, namely macular degeneration; retinal detachment; developmental and hereditary disorders; toxic, nutritional, and environmental disorders; inflammatory disorders; and diabetic retinopathy. With regard to other areas of vision research, the RPE exerts trophic influences on the development of ocular tissues and visual pathways, and is a factor to be considered in many types of intraocular surgery such as cataract extraction, vitrectomy, and retinal surgery. Within the broader field of biomedical research, the subjects of aging, genetics, toxicology, epidemiology, and epithelial cell transport bear upon the RPE.

SUBPROGRAM OBJECTIVES

- To gain fundamental knowledge about the retinal pigment epithelium in structural, molecular, and physiological terms.
- To understand the trophic influences of the RPE on other elements of the visual system.
- To determine the role of the RPE in chorioretinal disease and retinal detachment, both as a primary and secondary factor.
- To develop noninvasive methods for studying RPE function (see Chapter 13, "Noninvasive Techniques in the Study of Retinal Disorders").

OVERVIEW OF CURRENT RESEARCH SUPPORT

The National Eye Institute currently supports most of the research in the United States on visual cell and RPE interactions. In FY 1981, 24 individual grants were funded on this subject at a total cost of $1,931,000. Most of these deal with renewal phenomena and/or its diurnal and circadian aspects. The remainder deal with transport, general cell biology, biochemistry, physiology, or development.

Other Institutes of the National Institutes of Health provide some support for photoreceptor-RPE research not necessarily involving interaction between the two cell types. These are the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (chemistry and physiology), the National Institute of General Medical Sciences (development, cytoskeleton, cell membranes, visual cell function), the National Institute of Neurological and Communicative Disorders and Stroke (transduction), and the National Institute of Child Health and Human Development (cell recognition during development, taurine metabolism).

Other Federal agencies also provide significant support, most of it directed toward the effects of light and coherent radiation on the retina. These include the Veterans Administration, the Food and Drug Administration, Air Force, Army, and National Science Foundation.

Finally, two private foundations provide support: Fight for Sight, Inc., and the National Retinitis Pigmentosa Society.

With regard to strengths and gaps in research support, National Eye Institute funding in FY 1981 of individual grants dealing with the subject of visual cells and the pigment epithelium emphasized the subject of photoreceptor renewal phenomena and mechanisms. This subject includes, of course, the phagocytic role of the RPE in this process. Most of these grants dealt with either morphological or biochemical aspects of renewal, and most of these are primarily morphological in nature. RPE transport was the next most prominent subject of interest, emphasizing the transport of ions and metabolites. Only a few grants supported tissue culture, a subject that was emphasized in the last five-year Plan of the National Advisory Eye Council. A single grant was related to the subject of Bruch's membrane, and a few grants dealt with other metabolic features of the photoreceptors or RPE.

Emphasis on photoreceptor-RPE interactions is certainly appropriate. However, more emphasis should be placed on the molecular mechanisms of this interaction, and on transport processes between the RPE and retina. Because the transport of nutrients and metabolites entails passage of these compounds across the thin film of interphotoreceptor matrix interspersed between these tissues, the characterization and origin of this material is important. Emphasis should also be placed on the biochemical aspects of RPE function in general. In this regard, useful tissue culture models would be highly desirable as an adjunct to the use of RPE-choroid preparations, and would allow the study of RPE free of the complications introduced by choroidal elements. Furthermore, noninvasive methods for the testing of RPE function are needed for clinical purposes. The influence of environmental factors on RPE disease and aging is a subject which is also underemphasized.

RECENT ACCOMPLISHMENTS

Research interest in the RPE has been high in the past few years, in part because of encouragement from the previous five-year Plan, and because of exciting new developments in the field. A number of important facts have now been established about this cell layer. It is more apparent than ever that the RPE is a central element in the survival and function of the visual cells: it regulates the microenvironment, controls the flow of metabolites, maintains a close association of the two cell layers, plays a vital role in the rhodopsin cycle, absorbs excess light energy, and daily removes discarded photoreceptor debris.

Despite the lack of any cellular junctions between the photoreceptors and the RPE, the anatomical relationships between the two cell types are extraordinarily close and are, under normal circumstances, kept that way. Apical projections from the RPE envelop the tips of the rod and cone outer segments. In some species, the anatomical relationships between the RPE and cones are elaborate, with the RPE processes resembling the husk on an ear of corn [52] and the cone outer segment representing the ear proper. These relationships are apparently crucial. In the human retina, where cone outer segments frequently do not reach all the way to the RPE, these processes seek out and find the cone outer segments. [53] This close approximation, although less elaborate, is also seen between the RPE and rod outer segments. It may facilitate retinal adhesion and no doubt aids in the exchange of metabolites and other substances between the RPE and photoreceptors.

A close approximation to the photoreceptor outer segments is also important for the phagocytic role played by the RPE. [13] Rods shed portions of their outer segment tips shortly after the onset of light, [22–24] whereas cones perform this function after the light is extinguished. [25–27] Processes from the RPE somehow recognize these discarded fragments, surround and engulf them, and dispose of them by intracellular digestion. [13,17]

Over the course of a lifetime or following chronic nutritional deficiencies, partially digested outer segment fragments build up within the RPE cytoplasm, and the cells become engorged with lipofuscin granules. [35,36] Lipofuscin granules are a hallmark of aging cells. The RPE cells from animals that have been subjected to diets deficient in antioxidants such as vitamin E or selenium [54,55] also show a high content of lipofuscin granules. Antioxidants destroy harmful agents called free radicals, which build up in photoreceptors as a result of the light-trapping process. Free radicals cause the cross-linking of lipids in the outer segment membranes of rods and cones, [56] rendering them less digestible by the pigment epithelium. The result is a buildup of indigestible "clinkers" in the digestive furnaces of the RPE layer. This may render the RPE cells less effective in their general functions and may also secondarily cause defects in Bruch's membrane and the photoreceptors. [1,14,54]

The cytoskeletal elements [57,58] of the RPE apical surface are, no doubt, important for its phagocytic activities and may play a role in another exciting phenomenon. It is now apparent that the rod and cone outer segments are normally aligned in the retina to point toward the pupil of the eye. [59–61] Presumably, this positioning affords the photoreceptors maximum sensitivity to light because their photopigment molecules lie in the most direct path of unscattered light rays. The RPE may play a role in holding the outer segments in this favorable position. It has recently been demonstrated that such directional sensitivity is not present in humans whose photoreceptors have become detached from the RPE, or whose eye has been covered for some time. [62,63] The RPE is generously endowed with an intracellular network of tubules and filaments, the latter being capable of contraction much like their counterparts in muscle. [57,58] These components are almost certainly involved in phagocytosis and the positioning of melanin granules within the apical half of the RPE. They may play an active role in photoreceptor orientation as well. Likewise, they may be an important factor in the reestablishment of contacts between photoreceptors and RPE following retinal detachment and reattachment. [64]

The barrier and transport functions of the RPE have been fruitfully investigated in recent years. The RPE is part of the blood-ocular barrier which gives the retina a favored environment in contrast to that of certain other tissues of the body. [6] The anatomical substrate for the barrier is the RPE cell layer itself and a series of mutually continuous seals that extend like belts around small portions of the lateral surfaces of all RPE cells. Because of this barrier, proteins and many small molecules and ions cannot enter the neurosensory retina under normal circumstances. In pathological states such as inflammatory disease, diabetes mellitus, and certain retinal dystrophies, this barrier breaks down. [65]

Because of the tight junctions [6,7] between the RPE cells and striking asymmetries in the distribution of ion pumps [9,66] and passive ion conductances [7] on the apical and basal membranes, the RPE is capable of directing the flow of ions and water into or out of the subretinal space. Studies of isolated RPE-choroid preparations have shown that there is a Na^+-K^+ pump on the apical cell membrane. [8,9] This pump, along with the asymmetrical distribution of passive conductances to other ions, controls the ionic composition of the subretinal space. Because fluid movement is obligatorily coupled to salt

movement (that is, NaCl, $NaHCO^3$, and others), the state of hydration and therefore the size of the subretinal space is properly maintained, as is the intimate contact between photoreceptors and RPE. There is evidence that potassium ion (K^+) plays an important role in this transport. Photic stimulation of the retina produces a decrease in the K^+ concentration in the subretinal space that results from photoreceptor activity.[42] Such changes in K^+ can affect the magnitude of active metabolite and ion transport, and perhaps the volume of subretinal fluid.[67]

Because it is electrogenic, the Na^+–K^+ pump on the apical surface of the RPE[9,68] makes a large contribution to the voltage difference across the pigment epithelium and is a major component of standing potentials recorded at the cornea, thereby giving the clinical examiner a glimpse of the functional status of the RPE. This pump has been identified with the ouabain-sensitive Na^+–K^+ ATPase activity of the apical RPE membrane[33] and has been localized by autoradiography with ^{3}H-ouabain[34] (Figure 2).

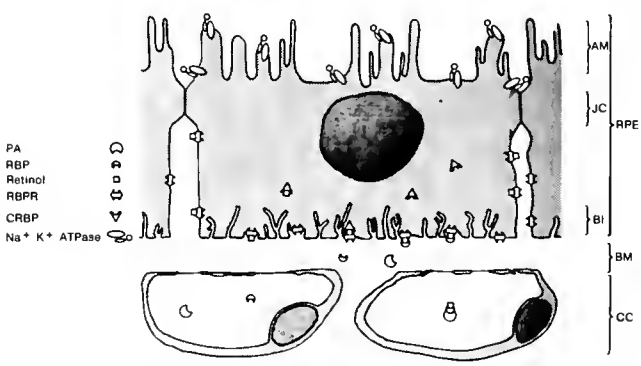


FIGURE 2. Diagram showing a current concept of retinal pigment epithelium (RPE) polarity and function with respect to two membrane proteins: the sodium-potassium pump (Na^+-K^+ ATPase) and the membrane receptor for retinol-binding protein (RBPR). Prealbumin (PA), retinol binding protein (RBP) and retinol circulate as a complex in the blood. This complex passes through the choroidal capillaries (CC), penetrates Bruch's membrane (BM) and recognizes the RBPR which are located on the basal infoldings (BI) and lateral plasma membrane of the RPE. The RBP releases retinol to the receptor and the retinol is somehow transported across the plasma membrane to a different retinal binding protein (CRBP) in the cytoplasm of the RPE. The RBP and PA dissociate and reenter the bloodstream. Sodium pumps are concentrated in the apical microvilli (AM) of the RPE where they, in concert with junctional complexes (JC), regulate cell volume and the ionic environment of photoreceptors. (Modified from Bok D, Autoradiographic Studies on the Polarity of Plasma Membrane Receptors in Retinal Pigment Epithelial Cells, Chapter 26, in Hollyfield, JG (ed): *The Structure of the Eye*, Vol IV, New York, Elsevier North Holland, Inc., 1982, p 254. Copyright 1982 by Elsevier Science Publishing Co, Inc. Reprinted by permission of the publisher.)

A dramatic aspect of RPE transport concerns its uptake of vitamin A (retinol) from the circulation and storage of vitamin A derivatives prior to transport to the photoreceptors where its aldehyde form (retinal) is used as the light-trapping chromophore of visual pigment. Vitamin A is transported from the liver to the eye by serum retinol binding protein (SRBP). The SRBP binds to receptor molecules on the basal (choroidal) surface of the RPE[69,70] and delivers the retinol to the interior of the cell while itself remaining extracellular. Nearly all the retinol in the RPE is stored as 11-*cis* and all-*trans* esters of palmitic and stearic acids.[71,72] Such stores possibly serve as reserves against dietary deprivation. In the cytosol, retinol and its derivatives (*retinoids*) are bound to cellular retinoid binding proteins names CRBP[73,74] (all-*trans* retinol), CRABP (retinoic acid) and CRA1BP[75] (11-*cis* retinal and 11-*cis* retinol). CRABP[76] is mainly confined to the retina, whereas the other two proteins occur in both retina and RPE. Although CRBP and CRABP occur in many cells, CRA1BP is not found elsewhere in the body. The role of these proteins in retinoid transport and isomerization during the visual cycle as well as in the routine functioning of the cell is not known. Similarly, the site and mechanism of isomerization of all-*trans* to 11-*cis* retinoid for visual pigment regeneration have not been established.

Retinol (in the all-*trans* conformation) is also delivered to the apical surface of the RPE subsequent to its formation in the ROS after rhodopsin has been bleached. A large protein in the interphotoreceptor matrix may be implicated in this transfer process.[77–79]

Molecular mechanisms underlying the recognition and ingestion of discarded outer segment tips during the photoreceptor renewal process have been difficult to study in intact animals. Therefore, in recent years, investigators have attempted to study this process in organ and tissue culture. The RPE of both embryonic[80] and adult animals (including humans)[81] will grow in artificial media, albeit with increasing difficulty as the RPE ages.[82] The human RPE can be cultured successfully for at least 40 hours after a person's death.[83] The RPE of the dystrophic rat retina retains its phagocytic defect in tissue culture.[84] Interestingly, normal cultured rat RPE cells favor the phagocytosis of light-exposed outer segment fragments over those that have been taken from dark adapted animals.[85] Their phagocytic activity is reduced in the presence of elevated levels of cyclic AMP.[86]

In spite of these interesting advances, the art and science of RPE culture needs considerable development. Optimum conditions for culture remain to be determined so that the cells can express as many of their in vivo features as possible. Most culture systems are deficient in this respect, with the result

that the cells lack essential items for their normal function, such as melanin granules and CRBP.[87]

Recently, an in vitro organ culture system was reported for photoreceptor shedding and RPE phagocytosis.[88] When the eyecups (dissected eyes lacking cornea, iris, and lens) of frogs are placed in an appropriate culture medium, normal rod shedding and phagocytosis occur in response to a light stimulus. One of the essential ingredients in this process is the proper concentration of bicarbonate ions. This is a most interesting and useful observation since bicarbonate is an essential element in RPE transport.[10] Using this culture system, perhaps the trophic interactions between RPE and photoreceptors regarding shedding and phagocytosis can now be studied in a systematic way.

As the above examples have demonstrated, the cellular properties of the RPE are beginning to be understood. Investigators would like to correlate them better with disease states in man. Because of its critical position within the eye, the RPE is implicated either primarily or secondarily in a wide range of ocular disorders. As more is learned about the precise manner in which RPE supports the photoreceptors, how specific genetic or acquired dysfunction of the RPE affects retinal viability, and how noninvasive tests monitor the function of the RPE, powerful tools will be available for the prevention and treatment of retinal disease.

RESEARCH NEEDS AND OPPORTUNITIES

One of the major accomplishments of the past decade has been the demonstration of the interdigitation between RPE and photoreceptor outer segments[11,12,15] and the discovery that the RPE phagocytizes and digests material from the tips of the continuously growing photoreceptor outer segments.[13] Nonetheless, many important questions remain unanswered. Among them: What changes occur in the "older" portions of the outer segments, and how is shedding ultimately triggered? Are humoral factors involved, and does the RPE play a role in the triggering? The recent demonstration that RPE phagocytosis of outer segment debris occurs in a cyclic fashion opens uncharted and potentially important territory to research. Phagocytosis occurs mostly in the morning for rod material[22–24] and at night for cone material,[25–27] following an internal (circadian) rhythm in rats[23] and light triggering in frogs.[24] Circumstantial evidence suggests that intraocular fluctuations in melatonin levels may play a role.[89] However, unequivocal evidence for the molecular mechanisms involved in shedding and the initial stages of phagocytosis remains to be found. The relationships between RPE apical processes and outer segments should also be explored. Additional questions are: What cytoskeletal elements are involved in RPE function?[90,91] When the retina detaches and reattaches, are the same connections reestablished, and to what extent does the RPE influence the restoration of retinal function? Does the RPE play an active role in outer segment orientation toward the pupil? In a normal eye, the outer segments angle towards the pupillary aperture to achieve maximum sensitivity to light (Stiles-Crawford effect of the first kind).[59–61] Recent studies have shown that this orientation is dynamic and may be altered by changing the location of the pupil,[62] or may change in the presence of retinal disease.[92] The role of the RPE in maintaining this direction system, or restoring it during the healing phase of disease, is of interest.

The RPE has many characteristics typical of other transporting epithelia such as that of the choroid plexus.[9,10] The cells are all joined by tight membrane junctions[6,7] which probably influence the passage of ions, amino acids, sugars, and proteins from one side of the RPE to the other.[93] Recent studies have begun to identify differences in the electrical transport properties of the apical and basal RPE membranes.[7,9,66–68] Most of the current data are from amphibians, but studies are now being done in mammals.[10] The data suggest the existence of highly specific transport systems for ions, glucose, taurine, and other metabolites important to the photoreceptors. Further research is needed to identify more transport processes, and answer questions, such as: What are the mechanisms for fluid transport across the RPE? What effects do drugs and nucleotides have on this process? This research has direct clinical implications.

The RPE, which is not itself sensitive to normal levels of environmental light, generates at least a portion of the c wave of the ERG[39–41] in response to changes in the extracellular potassium concentrations that occur after the photoreceptors respond to light.[42] Similar information is needed on the light peak of the standing potential, which forms the basis for the clinical EOG.[47,48] Direct recording of RPE responses in man has been difficult because of the slow time course and the small voltages involved. Further studies are important because these responses eventually may be used in the clinic to evaluate the integrity of the RPE. The fast photovoltage produced by the RPE[38] may prove useful for this purpose. Few clinically useful tests of RPE function exist at present, despite the great importance of this tissue to ocular function and disease.[46]

Adhesion between the retina and RPE is undoubtedly influenced by the anatomic relationships that have been mentioned and the existence of a viscous intercellular matrix.[28,29] However, recent

studies have shown that retinal adhesion falls dramatically within a few minutes after death or following experimental inhibition of oxidative metabolism by cyanide.[28–30] Thus, there appear to be active metabolic systems that are critical to retinal detachment. More research is needed to identify the specific physiologic mechanisms of adhesion. The subject of glycosaminoglycan synthesis[94,95] and function should be revived. Experiments involving pharmacologic modification of adhesion may result in clinical applications to the management of retinal detachments.

The barrier function of the RPE is of major importance in ocular pathology. Using techniques such as fluorescein angiography, vitreous fluorophotometry, and tracer histopathology (for example, with horseradish peroxidase), RPE barrier abnormalities have been found in disorders such as diabetes mellitus, central serous choroidopathy, pigment epitheliopathy, and retinitis pigmentosa.[65] Additional research is needed to determine why and how the barrier is affected, whether the changes occur within the cells or at the intercellular junctions, whether the barrier defects are limited to certain types of substrate, and whether such defects are causes or results of disease.

Work during the past several years has emphasized the role of the RPE in retinoid storage, utilization, and transport associated with the visual cycle and other cellular function.[69–71,73,74] These functions are critical to photoreception and are thought to be affected in a number of retinal diseases. Many questions remain. Information is needed on the controls that affect uptake, mobilization, and release of retinoids. Studies are also needed on the intracellular transport and utilization of retinoids in the visual cycle and on other roles that retinoids may play in the proper functioning of the cells. The role of the interphotoreceptor matrix in retinoid transport and the manner in which retinoid is released from and delivered to the apical surface of the RPE requires elucidation. The transport proteins involved in this process might be expected to have specific plasma membrane receptors on the RPE and outer segments.

There has been much concern of late about the effects of light on the physical components of the photoreceptors and RPE. The absorption of radiant energy, particularly in the shorter wavelengths, can lead to oxidation of lipids in the photoreceptor membrane, which renders them poorly digestible by the RPE.[54–56] These altered lipids are thought to contribute to the accumulation of autofluorescent pigments such as lipofuscin,[35] to the aging process within the RPE,[14,36,54] and most important, to the pathogenesis of macular degeneration. Much of this schema remains speculative, but lipofuscin unquestionably accumulates throughout life, and physical changes in the RPE and Bruch's membrane are characteristic of older and degenerated eyes.[14] Research is critically needed to probe the effects of light damage and sort out the intrinsic and extrinsic factors in aging, hereditary retinal degeneration, and macular degeneration. Epidemiological studies relating solar and artificial radiation intensities to the incidence of retinal disease might prove helpful. Understanding the relationship between light, natural antioxidants (selenium and vitamin E, for example), lipofuscin, and aging may lead to means for preventing aging-related maculopathy.

Some preliminary successes have been achieved in the study of RPE cells in laboratory culture.[80–82,84,85,87] Dystrophic rat RPE cells retain their phenotype, namely a depressed phagocytic function, in culture.[84] Normal rat RPE cells appear to choose light-exposed outer segment fragments over dark-exposed ones during phagocytosis.[85] Human RPE from individuals of various ages can also be cultured.[81,82] Studies with these cultures soon may be possible on the biochemical defects in inherited or degenerative disorders. However, before this can proceed systematically, more knowledge is needed about the conditions under which RPE cells from various sources, including humans, can be grown. To date, cultured RPE cells that most closely represent their in vivo counterparts in terms of morphology, biochemical aspects, and melanization are grown in specified medium from chicken embryos.[80,87] An equal or better level of success must be gained with RPE from other sources, including human material. In this connection, methods of acquiring human tissue must be improved. Donor programs, such as those being initiated for eyes with retinitis pigmentosa and diabetes, should be encouraged.

An additional means of extending the range of pathologic material available for study is the use of animal models. The primary site of mutant gene expression has now been localized with considerable confidence in the rdy rat (RPE)[21] and the rd mouse (photoreceptors).[96–98] There is good evidence that the gene for progressive retinal atrophy in Irish setters is expressed in the photoreceptors.[99] All animal models should be studied intensively until it becomes possible to retard or prevent photoreceptor degeneration. What is the most suitable model cannot be determined until there is more information both about the many available models and the various human diseases. The two subjects are intertwined; progress in one spurs progress in the other. Therefore, animal mutants with photoreceptor degeneration should be used to the maximum possible extent.

RECOMMENDATIONS

Based on the foregoing assessment of recent accomplishments, current activities, and research needs and opportunities in "Retinal Pigment Epithelium," the Panel has made the following recommendations concerning research in this subprogram over the next five years. These have been grouped under two headings: Program Base and Program Development Priorities.

The Program Base includes areas of ongoing research where the current level of activity is considered adequate, or areas of ongoing research in which there may be great need for additional activity, but where, in the Panel's judgment, little or no opportunity (new methods or insights) exists at present to justify a significant expansion of effort. Nonetheless, additional applications for research grants in these areas may be funded if they are innovative and of very high quality as determined by the NIH peer review system.

Program Development Priorities include areas of ongoing research in which new knowledge and techniques offer particular opportunities for scientific progress, or promising new areas of research in which there is little or no support at present but where there is both great need and high potential for success. Such areas are judged to warrant significantly increased support over the next five years, provided that high quality applications for research grants in these areas are forthcoming.

Program Base

- Investigate molecular mechanisms underlying the rhythmicity and control of RPE phagocytosis and metabolism.
- Determine the anatomical (that is, cytoskeletal elements) and functional (that is, outer segment orientation) relationships between photoreceptors and the retinal pigment epithelium, especially RPE ensheathment of photoreceptors following retinal detachment and reattachment.

Program Development Priorities

- Analyze special aspects of RPE transport, including that of vitamin A and its congeners (retinoids), ions, and metabolites. Characterize the biophysical and biochemical properties of the basolateral and apical membranes, and explore the possibility of retinal regional differences in these properties.
- Study the metabolism of the RPE with particular emphasis on cell isolation, culture, and fractionation methods. Explore the roles of calcium, nucleotides, hormones, retinoids, and other cell products in controlling the properties and functions of the RPE.
- Investigate the physiological and biochemical mechanisms of retinal adhesion and subretinal fluid resorption and their relationship to retinal detachment (see Chapter 6, "Retinal Detachment and Vitreous Disorders").
- Examine the barrier properties of the RPE and determine if these properties are altered in diseases of the retina and choroid (see Chapter 1, "Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities;" Chapter 4, "Developmental and Hereditary Disorders;" Chapter 5, "Macular Degeneration;" and Chapter 6).
- Examine the effects of light, age, drugs, and antioxidants on lipofuscin in the RPE cell. Relate these effects also to changes in Bruch's membrane, to the progress of inherited retinal degeneration, and to the development of macular degeneration (see Chapter 4, 5, and Chapter 9, "Photoreceptors, Visual Pigments, and Phototransduction").
- Improve noninvasive clinical tests for RPE function (see Chapter 13).
- Investigate the RPE as a potential source of antigens for autoimmune diseases of the retina (see Chapter 2, "Inflammatory Disorders").
- Identify, develop, and utilize animal models to study the cellular basis of RPE disease, especially as it relates to retinitis pigmentosa and macular degeneration (see Chapter 4, 5, and Chapter 14, "Tissue Acquisition and Distribution: Human Donor Eyes and Animal Models").

RESOURCE REQUIREMENTS

After reviewing current research grant support in each of these categories and assessing the need and potential for future development, the Panel has estimated the number of projects it believes are needed to carry out its recommendations in FY 1983. These estimates are shown in the table on the following page. For a discussion of the general basis and significance of these projections, see the "Summary" at the beginning of this report.

RESOURCE TABLE

RETINAL PIGMENT EPITHELIUM

	No. of Grants FY 1981	Panel Recommendation FY 83	
		Add. Grants	Total Grants
Program Base			
A. Investigate molecular mechanisms underlying the rhythmicity and control of RPE phagocytosis and metabolism.	9	0	9
B. Determine the anatomical and functional relationships between photoreceptors and the RPE.	3	0	3
Program Development Priorities			
A. Analyze special aspects of RPE transport. Characterize biochemical and biophysical properties.	6	3	9
B. Study the metabolism of the RPE with particular emphasis on cell isolation, culture, and fractionation methods. Explore the factors which control the properties and functions of the RPE.	2	2	4
C. Investigate the physiological and biochemical mechanisms of retinal adhesion and subretinal fluid resorption and their relationship to retinal detachment.	*	[2]*	*
D. Examine the barrier properties of the RPE and determine if these properties are altered in disease.	0	2	2
E. Examine the effects of light, age, drugs, and antioxidants on lipofuscin in the RPE cell.	3	2	5
F. Improve noninvasive clinical tests for RPE function.	1	3	4
G. Investigate the RPE as a potential source of antigens for autoimmune diseases of the retina.	0	1	1
H. Identify, develop, and utilize animal models to study the cellular basis of RPE disease.	0	[2]**	[2]**
Subtotal Grants	**24**	**13**	**37**
(% of Program)	**(6)**	**(12)**	**(8)**
Total Estimated Cost	**$1,931,000**	**$1,954,000**	**$3,885,000**

* Counted in Retinal Detachment and Vitreous Disorders subprogram. See Chapter 6.

** Counted in Developmental and Hereditary Disorders and Macular Degeneration subprograms. See Chapters 4 and 5.

REFERENCES

1. Hogan MJ, Alvarado JA, Weddell JE: *Histology of the Human Eye*. Philadelphia, WB Saunders Co, 1971, pp 328–363.
2. Newsome D, Kenyon K: Collagen production in vivo and in vitro by the retinal pigmented epithelium of the chick embryo. *Dev Biol* 32:387–400, 1973.
3. Friedman E, Smith TR, Kuwabara T: Senile choroidal patterns and drusen. *Arch Ophthalmol* 69:220–230, 1963.
4. Deutman AF, Jansen LMAA: Dominantly inherited drusen of Bruch's membrane. *Br J Ophthalmol* 54:373–382, 1970.
5. Farkas TG, Sylvester V, Archer D: The ultrastructure of drusen. *Am J Ophthalmol* 71:1196–1205, 1971.
6. Cohen AI: A possible cytological basis for the "R" membrane in the vertebrate eye. *Nature* 205:1222–1223, 1965.
7. Miller SS, Steinberg RH: Passive ionic properties of frog retinal pigment epithelium. *J Membr Biol* 36:337–372, 1977.
8. Steinberg RH, Miller S: Aspects of electrolyte transport in frog pigment epithelium. *Exp Eye Res* 16:365–372, 1973.
9. Miller SS, Steinberg RH, Oakley B: The electrogenic sodium pump of the frog retinal pigment epithelium. *J Membr Biol* 44:259–279, 1978.
10. Steinberg RH, Miller S: Transport and membrane properties of the retinal pigment epithelium, in Zinn KM, Marmor MF (eds): *The Retinal Pigment Epithelium*. Cambridge, Harvard University Press, 1979, pp 205–225.
11. Bairati A Jr, Orzolesi N: The ultrastructure of the pigment epithelium and the photoreceptor pigment epithelium junction in the human retina. *J Ultrastruct Res* 9:484–496, 1963.
12. Spitznas M, Hogan MJ: Outer segments of photoreceptors and the retinal pigment epithelium. *Arch Ophthalmol* 84:810–819, 1970.
13. Young RW, Bok D: Participation of the retinal pigment epithelium in the rod outer segment renewal process. *J Cell Biol* 42:392–403, 1969.
14. Hogan MJ: Role of the retinal pigment epithelium in macular disease. *Trans Am Acad Ophthalmol Otolaryngol* 76:64–80, 1972.
15. Hogan MJ, Wood I, Steinberg RH: Phagocytosis by pigment epithelium of human retinal cones. *Nature* 252:305–307, 1974.
16. Anderson DH, Fisher SK: Disc shedding in the rod-like and cone-like photoreceptors of tree squirrels. *Science* 187:953–955, 1976.
17. Bok D, Young RW: Phagocytic properties of the retinal pigment epithelium, in Zinn KM, Marmor MF (eds): *The Retinal Pigment Epithelium*. Cambridge, Harvard University Press, 1979, pp 148–174.
18. Herron WL, Riegel BW, Myers OE, et al: Retinal dystrophy in the rat: A pigment epithelial disease. *Invest Ophthalmol Vis Sci* 8:595–604, 1969.
19. Bok D, Hall MO: The etiology of retinal dystrophy in RCS rats. *Invest Ophthalmol Vis Sci* 8:649, 1969.
20. Bok D, Hall MO: The role of the pigment epithelium in the etiology of inherited retinal dystrophy in the rat. *J Cell Biol* 49:664–682, 1971.
21. Mullen RJ, LaVail MM: Inherited retinal dystrophy: Primary defect in pigment epithelium determined with experimental rat chimeras. *Science* 194:799–801, 1976.
22. LaVail MM: Rod outer segment disc shedding in relation to cyclic lighting. *Exp Eye Res* 23:277–280, 1976.
23. LaVail MM: Rod outer segment disc shedding in rat retina: Relationship to cyclic lighting. *Science* 194:1071–1074, 1976.
24. Basinger S, Hoffman R, Matthes M: Photoreceptor shedding is initiated by light in the frog retina. *Science* 194:1074–1076, 1976.
25. Young RW: The daily rhythm of shedding and degradation of cone outer segment membranes in the lizard retina. *J Ultrastruct Res* 61:172–185, 1977.
26. Young RW: The daily rhythm of shedding and degradation of cone outer segment membranes in the chick retina. *Invest Ophthalmol Vis Sci* 17:105–116, 1977.
27. O'Day WT, Young RW: Rhythmic daily shedding of outer segment membranes by visual cells in the goldfish. *J Cell Biol* 76:593–604, 1978.
28. De Guillebon H, Zauberman H: Experimental retinal detachment: Biophysical aspects of retinal peeling and stretching. *Arch Ophthalmol* 87:545–598, 1972.
29. Zauberman H: Adhesive forces between the retinal pigment epithelium and sensory retina, in Zinn KM, Marmor MF (eds): *The Retinal Pigment Epithelium*. Cambridge, Harvard University Press, 1979, pp 192–204.
30. Marmor MF, Abdul-Rahim AS, Cohen DS: The effect of metabolic inhibitors on retinal adhesion and subretinal fluid resorption. *Invest Ophthalmol Vis Sci* 19:893–903, 1980.
31. Curran PF: Sodium, chloride and water transport by rat ileum in vitro. *J Gen Physiol* 43:1137–1148, 1960.
32. Diamond JM: The mechanism of water transport by the gall bladder. *J Physiol (Lond)* 161:503–527, 1962.
33. Ostwald TJ, Steinberg RH: Localization of frog retinal pigment epithelium Na^+–K^+ ATPase. *Exp Eye Res* 31:351–369, 1980.
34. Bok D: Autoradiographic studies on the polarity of plasma membrane receptors in retinal pigment epithelial cells, in Hollyfield J (ed): *IV International Symposium on the Structure of the Eye*. New York, Elsevier-North Holland, pp 245–256, 1982.
35. Streeten BW: Sudanophilic granules of the human retinal pigment epithelium. *Arch Ophthalmol* 66:391–398, 1961.
36. Feeney L, Grieshaber JA, Hogan MJ: Studies on the human ocular pigment, in Rohen JW (ed): *The Structure of the Eye*. Stuttgart, FK Schattauer-Verlag, 1965, pp 535–548.
37. Reme CE: Autophagy in visual cells and pigment epithelium. *Invest Ophthalmol Vis Sci* 16:807–814, 1977.

38. Ebrey TG, Cone RA: Melanin, a possible pigment for the photostable electrical responses of the eye. *Nature* 213:360–362, 1967.
39. Noell WK: The origin of the electroretinogram. *Am J Ophthalmol* 38:78–90, 1954.
40. Brown KT, Wiesel TN: Localization of origins of electroretinogram components by intraretinal recording in the intact cat eye. *J Physiol (Lond)* 158:257–280, 1961.
41. Steinberg RH, Schmidt R, Brown KT: Intracellular responses to light from cat pigment epithelium: Origin of the electroretinogram c-wave. *Nature* 227:728–730, 1970.
42. Oakley B, Green DG: Correlation of light-induced changes in retinal extracellular potassium concentration with the C-wave of the electroretinogram. *J Neurophysiol* 39:1117–1133, 1976.
43. Oakley B II: Potassium and the photoreceptor-dependent pigment epithelial hyperpolarization. *J Gen Physiol* 70:405–425, 1977.
44. Noell WK: Azide-sensitive potential difference across the eye-bulb. *Am J Physiol* 170:217–238, 1952.
45. Noell WK, Crapper DR, Paganelli CV: Transretinal currents and ion fluxes, in Snell FM, Noell WK (eds): *Transcellular Membrane Potential and Ionic Fluxes*. New York, Gordon & Breach, 1964, pp 92–130.
46. Marmor MF, Lurie M: Light-induced electrical responses of the retinal pigment epithelium, in Zinn KM, Marmor MF (eds): *The Retinal Pigment Epithelium*. Cambridge, Harvard University Press, 1979, pp 226–244.
47. Griff ER, Steinberg RH: Origin of the light peak: In vitro study of *Gekko gekko*. *J Physiol*, 1982, to be published.
48. Linsenmeier RA, Steinberg RH: Origin and sensitivity of the light peak in the intact cat. *J Physiol*, 1982, to be published.
49. Parry HB: Degeneration of the dog retina: VI. Central progressive atrophy with pigment epithelial dystrophy. *Br J Ophthalmol* 38:653–668, 1954.
50. Barnett K, Dunn W: The International Sheep Dog Society and progressive retinal atrophy. *J Small Anim Pract* 10:301, 1969.
51. Aguirre G, Rubin L: Diseases of the retinal pigment epithelium in animals, in Zinn KM, Marmor MF (eds): *The Retinal Pigment Epithelium*. Cambridge, Harvard University Press, 1979, pp 334–356.
52. Steinberg RH, Wood I: Pigment epithelial ensheathment of cone outer segments in the retina of the domestic cat. *Proc R Soc Lond (Biol)* 187:461–478, 1974.
53. Steinberg RH, Wood I, Hogan MS: Pigment epithelial ensheathment and phagocytosis of extrafoveal cones in the human retina. *Philos Trans R Soc Lond (Biol)* 277: 459–474, 1977.
54. Hayes KC: Retinal degeneration in monkeys induced by deficiencies of vitamin A or E. *Invest Ophthalmol Vis Sci* 13:499–510, 1974.
55. Katz ML, Stone WL, Dratz EA: Fluorescent pigment accumulation in retinal pigment epithelium of antioxidant deficient rats. *Invest Ophthalmol Vis Sci* 17:1049–1058, 1978.
56. Farnsworth CC, Dratz EA: Oxidative damage of retinal rod outer segment membranes and the role of vitamin E. *Biochim Biophys Acta* 443:556–570, 1976.
57. Murray RL, Dubin MW: The occurrence of actin-like filaments in association with migrating pigment granules in frog retinal pigment epithelium. *J Cell Biol* 64:705–710, 1975.
58. Burnside B, Laties AM: Actin filaments in apical projections of the primate pigmented epithelial cell. *Invest Ophthalmol Vis Sci* 15:570–575, 1976.
59. Laties AM: Histochemical techniques for the study of photoreceptor orientation. *Tissue Cell* 1:63–81, 1969.
60. Laties A, Liebman P, Campbell C: Photoreceptor orientation in the primate eye. *Nature* 218:172–173, 1968.
61. Laties AM, Enoch JM: An analysis of retinal receptor orientation: Angular relationship of neighboring photoreceptors. *Invest Ophthalmol Vis Sci* 10:69–77, 1971.
62. Enoch JM, Birch DG: Evidence for alteration in photoreceptor orientation. *Ophthalmology* 87:821–833, 1980.
63. Enoch JM, Birch DG, Birch EE, et al: Effect of uniocular occlusions on selected visual functions. *Trans Ophthalmol Soc UK* 99:407–412, 1980.
64. Steinberg RH, Wood I: The relationship of the retinal pigment epithelium to photoreceptor outer segments in the human retina, in Zinn KM, Marmor MF (eds): *The Retinal Pigment Epithelium*. Cambridge, Harvard University Press, 1979, pp 32–44.
65. Cunha-Vaz JG: *The Blood-Retinal Barriers*. New York, Plenum Press, 1980.
66. Miller SS, Steinberg RH: Active transport of ions across frog retinal pigment epithelium. *Exp Eye Res* 25:235–248, 1977.
67. Miller SS, Steinberg RH: Potassium modulation of taurine transport across the frog retinal pigment epithelium. *J Gen Physiol* 74:237–259, 1979.
68. Oakley B, Miller SS, Steinberg RH: Effect of intracellular potassium upon the electrogenic pump of frog retinal pigment epithelium. *J Membr Biol* 44:281–307, 1978.
69. Heller J, Bok D: A specific receptor for retinal binding protein as detected by the binding of human and bovine retinol binding protein to pigment epithelial cells. *Am J Ophthalmol* 81:93–97, 1976.
70. Bok D, Heller J: Transport of retinol from the blood to the retina: An autoradiographic study of the pigment epithelial cell surface receptor for plasma retinol binding protein. *Exp Eye Res* 22:395–402, 1976.
71. Dowling JE: Chemistry of visual adaptation in the rat. *Nature* 188:114–118, 1960.
72. Bridges CDB: Storage distribution and utilization of vitamin A in the eyes of adult amphibians and their tadpoles. *Vision Res* 15:1311–1323, 1975.
73. Wiggert BO, Chader GJ: A receptor for retinol in the developing retina and pigment epithelium. *Exp Eye Res* 21:143–151, 1975.

74. Saari JC, Futterman S: Retinol-binding protein in bovine retina: Isolation and partial characterization. *Exp Eye Res* 22:425–434, 1976.
75. Futterman S, Saari JC, Blair S: Occurrence of a binding protein for 11-*cis*-retinal in retina. *J Biol Chem* 252:3267–3271, 1977.
76. Futterman S, Saari JC, Swanson DE: Retinol and retinoic acid-binding proteins in bovine retina: Aspects of binding specificty. *Exp Eye Res* 22:419–424, 1976.
77. Adler A, Severin KM: Proteins of the bovine interphotoreceptor matrix: Tissues of origin. *Exp Eye Res* 32:755–769, 1981.
78. Liou GI, Bridges CDB, Fong S-L: Vitamin A transport between retina and pigment epithelium: An interphotoreceptor matrix protein carrying endogenous retinol (IRBP). *Invest Ophthalmol Vis Sci* 22(suppl):65, 1982.
79. Lai YL, Wiggert B, Lin YP, et al: Interphotoreceptor retinol-binding proteins: Possible transport vehicles between compartments of the retina. *Nature*, 1982, to be published.
80. Newsome D, Fletcher R, Robison W, et al: Effects of cyclic AMP and Sephadex fractions of chick embryo extract on cloned retinal pigment epithelium in tissue culture. *J Cell Biol* 61:369–382, 1974.
81. Albert DM, Tso MOM, Robson AS: In vitro growth of pure cultures of retinal pigment epithelium. *Arch Ophthalmol* 88:63–69, 1972.
82. Flood MT, Gouras P, Kjeldbye H: Growth characteristics and ultrastructure of human retinal pigment epithelium in vitro. *Invest Ophthalmol Vis Sci* 19:1309–1320, 1980.
83. Edwards RB: Culture of mammalian retinal pigment epithelium and neural retina, in Packer L (ed): *Methods in Enzymology*. New York, Academic Press, pp 39–43, 1982.
84. Edwards RB, Szamier RB: Defective phagocytosis of isolated rod outer segments by RCS rat retinal pigment epithelium in culture. *Science* 197:1001–1003, 1977.
85. Hall MO: Phagocytosis of light- and dark-adapted rod outer segments by cultured pigment epithelium. *Science* 202:526–528, 1978.
86. Edwards RB, Bakshian S: Phagocytosis of outer segments by cultured rat pigment epithelium. *Invest Ophthalmol Vis Sci* 19:1184–1188, 1980.
87. Israel P, Masterson E, Goldman AI, et al: Retinal pigment epithelial cell differentiation in vitro: Influence of culture medium. *Invest Ophthalmol Vis Sci* 19:720–727, 1980.
88. Besharse JC, Terrill RO, Dunis DA: Light evoked disc shedding by rod photoreceptors in vitro. *Invest Ophthalmol Vis Sci* 19:1512–1517, 1980.
89. Pang SF, Brown GM, Grota LJ, et al: Determination of N-Acetylserotonin and melatonin activities in the pineal gland, retina, harderian gland, brain and serum of rats and chickens. *Neuroendocrinology* 23:1–13, 1977.
90. Blank K, Provine RR, Enoch JM: Shift in the peak of the photopic Stiles-Crawford function with marked accommodation. *Vision Res* 15:499–508, 1975.
91. Laties AM, Burnside B: The maintenance of photoreceptor orientation, in Nachmias PF, Sanger JW (eds): *Motility and Cell Function: Proceedings of the First John M. Marshall Symposium in Cell Biology*. New York, Academic Press, 1979, pp 285–298.
92. Fitzgerald CR, Enoch JM, Birch DG, et al: Anomalous pigment epithelial photoreceptor relationships and receptor orientation. *Invest Ophthalmol Vis Sci* 19:956–966, 1980.
93. Miller SS, Steinberg RH: Transport of taurine, L-methionine and 3–0- methyl-D-glucose across frog retinal pigment epithelium. *Exp Eye Res* 23:177–189, 1976.
94. Berman ER: The biosynthesis of mucopolysaccharides and glycoproteins in pigment epithelial cells of bovine retina. *Biochim Biophys Acta* 83:371–373, 1964.
95. Ocumpaugh DE, Young RW: Distribution and synthesis of sulfated mucopolysaccharides in the retina of the rat. *Invest Ophthalmol Vis Sci* 5:196–103, 1966.
96. Schmidt SY, Lolley RN: Cyclic nucleotide phosphodiesterase: An early defect in inherited retinal degeneration of C3H mice. *J Cell Biol* 57:117–123, 1973.
97. Farber DB, Lolley RN: Cyclic guanosine monophosphate: Elevation in degenerating photoreceptor cells of the C3H mouse retina. *Science* 186:449–551, 1974.
98. LaVail MM, Mullen RJ: Role of the pigment epithelium in inherited retinal degeneration analyzed with experimental mouse chimeras. *Exp Eye Res* 23:227–245, 1976.
99. Aguirre G, Farber D, Lolley R, et al: Rod-cone dysplasia in Irish setters: A defect in cyclic GMP metabolism in visual cells. *Science* 201:1133–1134, 1978.

9

PHOTORECEPTORS, VISUAL PIGMENTS, AND PHOTOTRANSDUCTION

INTRODUCTION

THE RETINAL PHOTORECEPTOR cells, the rods and cones, are biological transducers that convert the light absorbed by their visual pigment molecules into neural (electrochemical) signals. Complex ionic and enzymatic processes greatly amplify photoreceptor responses; other ionic and feedback mechanisms modify the size and shape of the signals as they are conveyed to the photoreceptor's synaptic terminals, where the visual message is transmitted to adjacent bipolar and horizontal cells for further processing.

In evolving to perform these tasks, rods and cones have become both the most highly differentiated and the most metabolically active cells in the visual pathway. They are frequently the first cells in the visual pathway to degenerate or die from hereditary defects such as retinitis pigmentosa, overexposure to light, toxic agents, and dietary inadequacies such as vitamin A deficiency. Moreover, the rate at which photoreceptor cells age may play a key role in initiating aging-related maculopathy, which is a leading cause of blindness.

Decades of research have produced a broad outline of the primary physiological roles and mechanisms of photoreceptors. Indeed, more is known about the role, structure, and physiology of rods and cones than about any other type of cell in the brain. Like other neurons, photoreceptors are unable to replace themselves by cellular division once they have become specialized, and they rely totally for rejuvenation upon elaborate mechanisms for replacing worn-out cellular components. Recently, many investigators have redirected their research on physiological functions to focus more on metabolic processes, especially the mechanisms by which the cells maintain, repair, and renew their structures. Elegant studies have revealed many of the details of how photoreceptors renew their outer segments by continuously inserting newly synthesized molecules of visual pigment near the ciliary "root" and discarding older "worn out" molecules by periodically shedding the tips of the outer segments. The shed fragments are then phagocytized and digested by the retinal pigment epithelium cells, which surround, protect, and interact with the outer segments. An understanding of these mechanisms may point the way to preventing or arresting the degenerations of photoreceptors that underlie many leading causes of blindness, such as aging-related maculopathy and retinitis pigmentosa.

The photoreceptors interact strongly with the cells they innervate in the neural retina, and they depend vitally on the cells of the retinal pigment epithelium, which provide most of their metabolic support and remove and process most of their wastes and debris. Disruption of this metabolic relationship can have severe clinical implications. Thus, the research in this subprogram, which is directed largely at gaining a basic understanding of the structure, physiology, and metabolism of the photoreceptors, greatly overlaps the concerns of many other Retinal and Choroidal Diseases subprograms.

SUBPROGRAM OBJECTIVES

- To determine the molecular structure and functions of photoreceptor macromolecules, membranes, and organelles.
- To elucidate the metabolic mechanisms by which photoreceptors maintain, repair, and renew their structures.
- To characterize the major physiological functions of photoreceptors (transduction, adaptation, and synaptic interactions) in terms of the underlying molecular mechanisms.
- To identify the structural and functional differences between rods and cones, particularly as they relate to diseases that affect one or the other cell type.
- To discover the genetic factors and cell interactions that control photoreceptor growth, differentiation, and maintenance.
- To discover the causes of photoreceptor degeneration and develop methods for preventing or slowing these processes and facilitating the recovery of photoreceptor function after injury (see Chapter 4, "Developmental and Hereditary Disorders" and Chapter 5, "Macular Degeneration").
- To develop and use noninvasive techniques for better assessing photoreceptor function in humans (see Chapter 13, "Noninvasive Techniques in the Study of Retinal Disorders").

OVERVIEW OF CURRENT RESEARCH SUPPORT

The National Eye Institute supports about three-fourths of all research on photoreceptors performed in the United States. Other Federal agencies support about one-fifth of all such research, and private foundations support most of the remainder. In FY 1981, the National Eye Institute funded 112 research projects in this subprogram at a total cost of $9,514,000. The focus of the projects supported by the National Eye Institute concerned the normal physiological function of photoreceptors, the photochemistry and structure of rhodopsin and disc membranes, and the metabolic processes that maintain photoreceptors. The projects supported by the Air Force and Army emphasized laser effects on photoreceptor structure and physiology.

Overall, studies of photoreceptor physiology and rhodopsin structure and function were well-supported and were making excellent progress. Indeed, much of the highest quality and most advanced research that is sponsored by the National Eye Institute is in this area. More support is needed for research on photoreceptor metabolism, especially for studies of the molecular mechanisms of synthesis, repair, and renewal, and investigations of the environmental and nutritional effects on photoreceptor degeneration and aging.

RECENT ACCOMPLISHMENTS

For many years, it had been thought that light damages the retina only when it is intense enough to heat and burn the tissue. However, about 16 years ago Noell and his colleagues showed that albino rats can become blinded by ordinary room lighting.[1] Subsequent studies confirmed this finding and helped to characterize the light intensities, wavelengths, and exposure times that can damage the photoreceptors and retinal pigment epithelium in rats, monkeys, and many other animals.[2–4] Blue light is more damaging to the photoreceptors than longer wavelength light,[5] and in primates, cones appear more sensitive to light toxicity than rods.[6] The light levels needed to damage primate photoreceptors are not extraordinary; monkeys with dilated pupils who have been surrounded by daylight fluorescent lamps for 12 hours have markedly disrupted rod and cone outer segments throughout the macular region of the retina[7] (Figure 1).

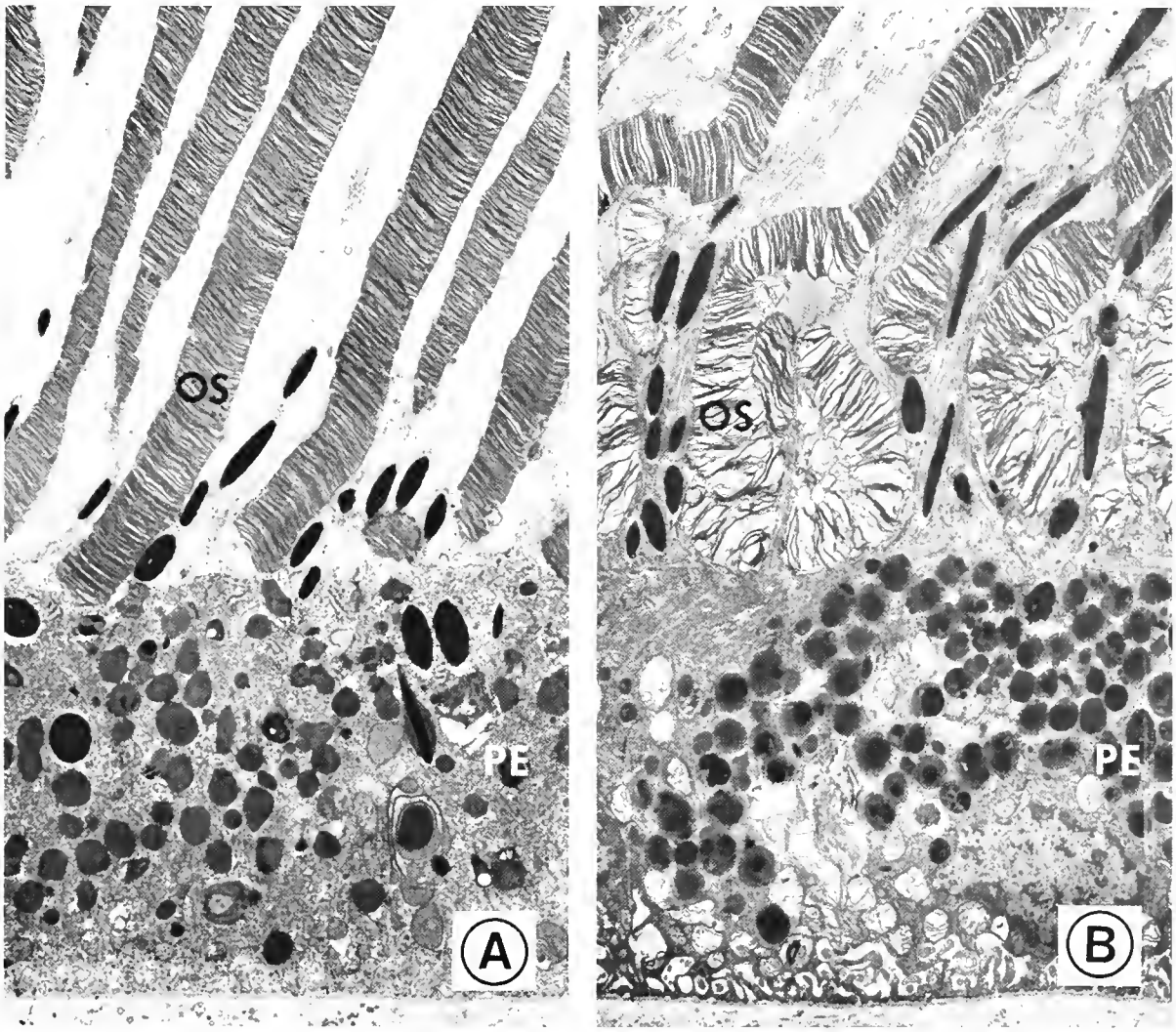


FIGURE 1. Retinal pigment epithelium (PE) and distal portions of photoreceptor outer segments (OS) of the macula in a retina. (A) From patched eye. (B) From eye exposed to 24,700 lux. Only rod outer segments are shown in the micrograph of the exposed eye. Vacuoles associated with membrane lamellae in the PE were distributed irregularly in both exposed and unexposed retinas (X7100). (From Sykes SM, Robison WG, Jr, Waxler M, et al: Damage to the monkey retina by broad spectrum fluorescent light. *Invest Ophthalmol Vis Sci* 20:425434, 1981).

Intermittent intense light can be even more damaging to photoreceptors than continuous light, and blue-sensitive cones in the monkey can be selectively killed by intermittent exposures to intense blue light.[6] Continuous rather than intermittent exposure to blue light produces extensive damage of the retinal pigment epithelium, but little photoreceptor degeneration. These studies suggest that the blue-absorbing chromophores from bleached or bleaching visual pigments may help mediate this phototoxicity.

Several investigators have begun to examine whether the intense lights often used during surgery might contribute to the incidence and severity of chronic cystic maculopathy that can follow cataract operations.[8–11] This concern was discussed at a symposium sponsored by the National Eye Institute on "Intense Light Hazards in Ophthalmic Diagnosis and Treatment," which helped to outline the type of research needed to resolve this important question.[4]

The growing awareness of the damaging effects of blue and near-ultraviolet light on the retina and retinal pigment epithelium is related to a growing interest in the damage to photoreceptors produced by oxidation and free radicals. Because the photoreceptors necessarily contain pigments to absorb light and their membranes have a very high content of polyunsaturated fatty acids, these cells may be

unusually susceptible to damage by lipid peroxidation. Blue and near-ultraviolet light can readily produce such peroxides, as can high levels of oxygen. Rods and cones contain large amounts of vitamin E and other antioxidants, which may help protect them against the inevitable damage sustained while performing their normal function of detecting light.[12–16] The questions that have thus emerged are: Does blue and near-ultraviolet light accelerate aging and degeneration in the retina and retinal pigment epithelium? Do aging-related maculopathy or other retinal degenerations result from or are they exacerbated by toxic effects of light exposure?[17]

Major advances have been made in techniques for culturing cells and tissues that promise to facilitate greatly research on photoreceptors. For example, it has been shown that photoreceptors can continue the shedding process in an eyecup culture[18] and that tissue cultures of isolated retinal pigment epithelium can continue to phagocytize shed fragments of outer segments in the absence of the retina.[19,20] Thus, both the shedding and the phagocytosis mechanisms can be studied in tissue cultures, where these mechanisms can be manipulated with drugs as well as with light, and also can be more readily analyzed biochemically. Moreover, it may be possible soon to culture these tissues from diseased human eyes and thereby gain direct access to the cellular and molecular dysfunctions involved in the disease process.

The most exciting advance in photoreceptor physiology during the past few years has been the rapid progress in elucidating the mechanisms of excitation and adaptation. (For a recent summary see reference 21.) Single photon responses of rod outer segments have now been detected.[22–25] For more than 30 years, it has been known that rods can reliably detect single photons of light, and during the last 15 years, numerous invertebrate photoreceptors have been shown to produce "quantum bumps" (large transient membrane currents that are the end result of the powerful amplifying mechanism a photon of light initiates on being caught by a rhodopsin molecule). (Figure 2).

A highly useful new technique[22] for studying single photon responses consists of sucking an individual rod outer segment into a close-fitting glass micropipette. The ionic current that flows extracellularly from the outer segment to the inner segment can thereby be monitored. This ionic current, pumped out of the cell by Na^+ pumps in the inner segment, flows continuously back into the cell through Na^+ channels located in the plasma membrane surrounding the outer segment. The effect of light is to diminish or block this continuous "dark" current by blocking the Na^+ channels. In a dark-adapted cell, the absorption of one photon by a rhodopsin molecule triggers an excitatory amplification mechanism that can transiently block about 1 percent of all the Na^+ channels, which in turn blocks the entry of some 10^7 Na^+ ions.[26,27] It is this transient reduction in Na^+ current that can be detected directly using the suction electrode.

This new technique has opened the way for a wide range of studies of the excitatory and adaptation mechanisms. For example, rods sporadically produce spontaneous (thermal) responses in the dark whose rate of occurrence closely matches the intensity of the psychophysically inferred "dark light" that sets the limit of human visual sensitivity in a fully dark adapted subject.[28–31] These responses are probably generated by spontaneous (thermal) isomerizations of the chromophore of rhodopsin. Because there are more than 10^8 rhodopsin molecules per human rod, such thermal isomerizations must be exceedingly infrequent, occurring at a rate of once every 1,000 years per rhodopsin molecule. This great stability of the chromophore against thermal isomerization when it is bound to the protein (opsin), together with the high probability that it will isomerize when it does catch a photon, is the molecular basis for the remarkable ability of photoreceptors to detect single photons of light reliably and efficiently.

The way in which the protein (opsin) can both stabilize the chromophore against thermal isomerization and yet enable the chromophore to isomerize efficiently after it catches a photon is not yet known. However, rapid progress has been made in understanding the molecular details that regulate the wavelength sensitivity of visual pigments as well as the mechanism of photoisomerization. The use of chemically modified analogues of retinal, together with theoretical computations, has permitted the development of a detailed model for the way in which charged groups in the protein can alter the peak absorption wavelength of the chromophore.[32,33]

The photoisomerization process has been clarified by employing Raman spectroscopy, high-speed flash photometry, and deuterated analogues of retinal. The result has been to verify and refine the original cis-trans isomerization process proposed by Hubbard and Kropf.[34] It now appears that the first spectral transition in the rhodopsin cycle, the formation of bathorhodopsin, represents the conversion of the chromophore to a twisted all-trans configuration.[35] Photocalorimetric measurements indicate that more than 60 percent of the energy of the photon is stored by the chromophore in this twisted configuration.[36] The twisted configuration

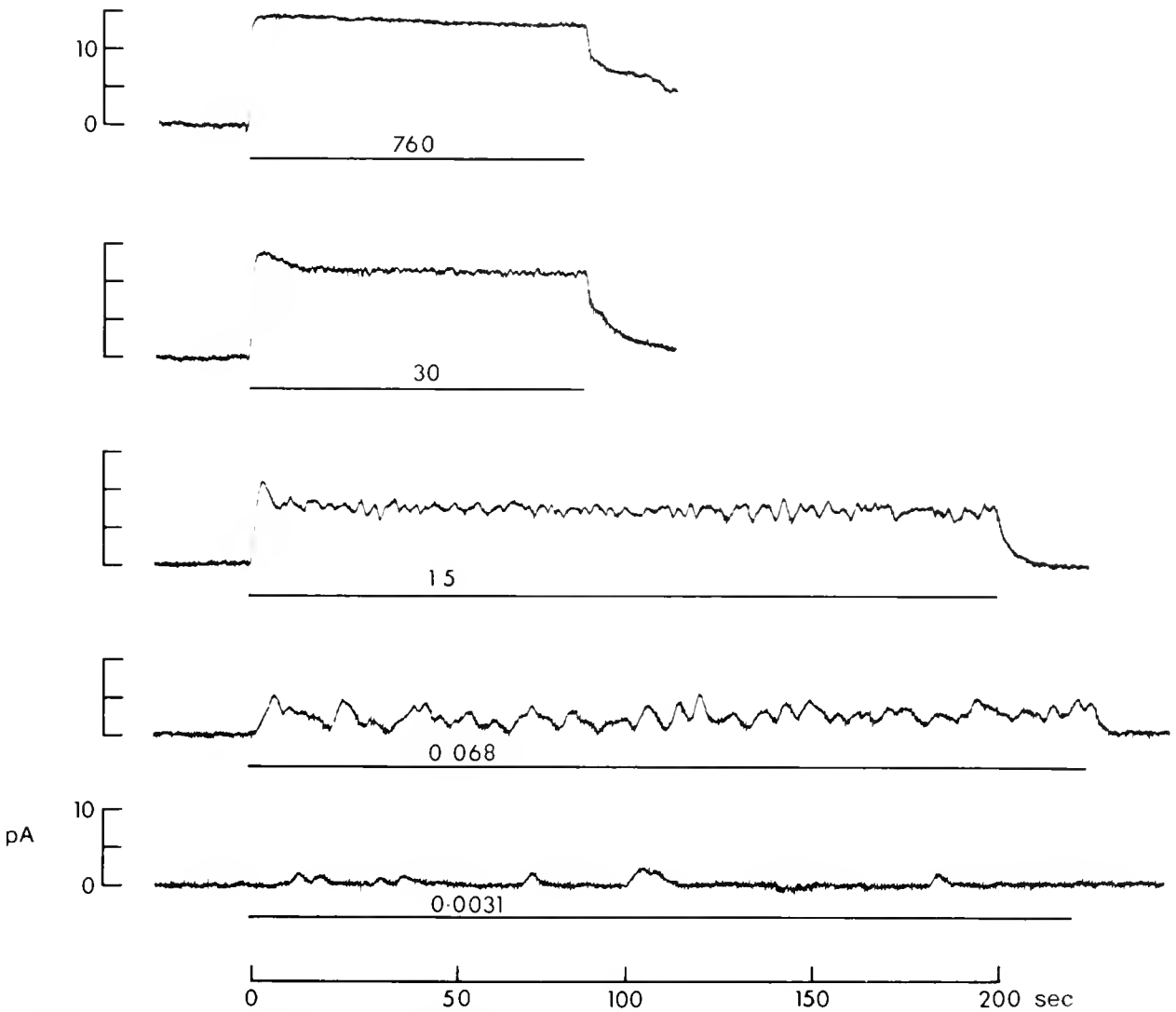


FIGURE 2. Response of a rod outer segment to steady light of different intensities as recorded with a suction electrode. Single photon responses are evident at the lowest light intensities (photon μm^{-2} sec^{-1} of 500 nm light). (From Baylor DA, Lamb TD, Yau K-W: *J Physiol* 288:613-635, 1979.)

relaxes as a later stage in the cycle (meta I) is reached.[37] The isomerization of the chromophore clearly induces some small changes in the conformation of the protein,[38-42] and at least one probable role for these conformational changes is to activate various enzymes.

Rhodopsin is now known to activate at least three different enzymes, a GTPase,[43-45] a phosphodiesterase,[46,47] and a kinase.[48,49] Rapid progress is being made in characterizing these enzymes, their roles, and their interactions. (For a recent overview see reference 21.) Current evidence indicates that a photoactivated rhodopsin molecule may transiently bind a GTP-binding protein complex which is otherwise free to diffuse on the membrane surface. After interacting with the rhodopsin, this complex appears to activate the phosphodiesterase.[43-45] Phosphodiesterase, in turn, rapidly hydrolyzes cGMP, significantly lowering the local concentration of cGMP in the outer segment. The kinase activated by rhodopsin is the slowest acting of the three enzymes.[48] It phosphorylates rhodopsin and thus may play a role in turning off the excitatory process or participate in an adaptation process.

There is evidence that the enzyme cascade, which lowers the cGMP concentration, is involved directly in the excitation pathway, making cGMP one of the internal messengers that regulates the Na^+ channels (Figure 3). However, a recent observation[50] indicates that the rate at which the cGMP level falls after flash stimulation may be too slow for cGMP to act as an excitatory signal. Thus, the precise role of the enzyme cascade remains to be established.

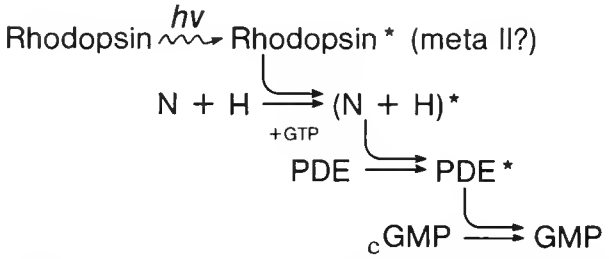


FIGURE 3. Enzyme cascade activated by rhodopsin that lowers cGMP level in rod outer segments. (N is a GTP-binding protein, H is a 'helper' protein, PDE is a phosphodiesterase).

New evidence has been found to support the claim that calcium ion (Ca^{++}) is at least one of the excitatory transmitters released by rhodopsin. Yoshikami and Hagins proposed this role for Ca^{++} over ten years ago, but despite intensive efforts the light-activated release of calcium ions from rod discs required by this hypothesis was not demonstrated unequivocally.[51] However, by placing a retina on the surface of a Ca^{++} sensitive electrode, investigators have recently discovered that large quantities of Ca^{++} are released from the outer segments (into the extracellular space) immediately following flash illumination: a single rhodopsin molecule can initiate the release of more than 10^4 Ca^{++} ions from the outer segment.[52] Moreover, other investigators, have shown that the time course of the Ca^{++} release can, under some conditions, closely match the time course of the receptor potential.[53] Because cytoplasmic Ca^{++} can block the Na^+ channels,[54–56] it appears likely that rhodopsin somehow initiates the release of a large amount of Ca^{++} into the cytoplasm. Whether this Ca^{++} is released from inside the discs is still unknown, and the possibility that cytoplasmic Ca^{++} is enzymatically mobilized remains to be examined. The recently discovered Na^+/Ca^{++} exchanger in the plasma membrane is probably responsible for pumping the Ca^{++} out of the outer segment[57,58] (Figure 4).

Thus, although many details remain to be worked out, the burst of discoveries of these past few years suggests that many of the persistent fundamental questions concerning phototransduction will soon

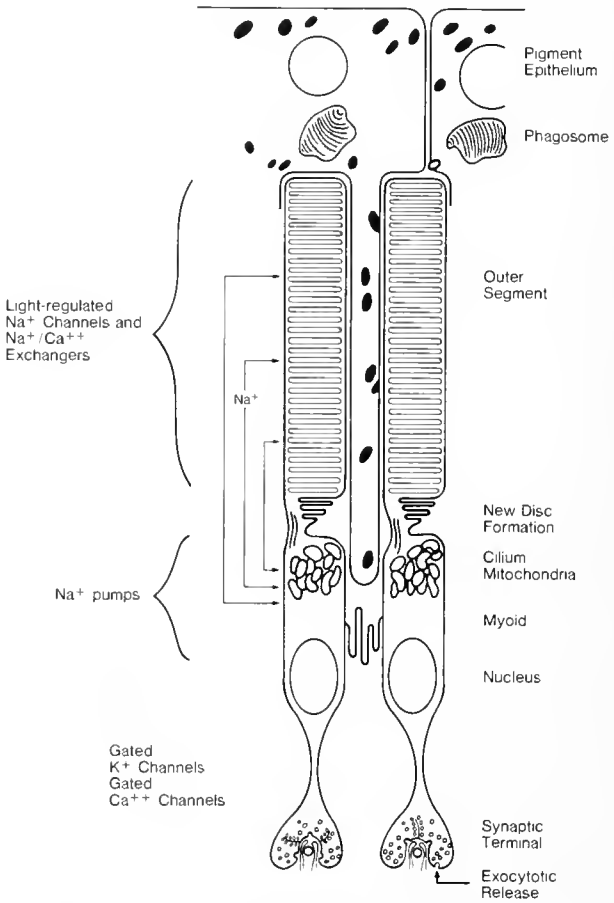


FIGURE 4. Diagram to illustrate some organelles of the rod photoreceptor and the approximate distribution of some of the ion channels, pumps, and exchangers in the plasma membrane.

be answered. The ion/nucleotide/ enzyme systems now being investigated in photoreceptors are employed by all types of eukaryotic cells in performing their physiological functions; thus, the rapidly progressing research on rods and cones should have broad application to vision research and cell physiology in general.

Substantial progress continues to be made in investigations of photoreceptor structure, biochemistry, and metabolism—basic research accomplishments that will be needed for a full understanding of how the photoreceptors are maintained in a healthy state and how they are perturbed by pathological processes that end their normal function. The carbohydrate structure of rhodopsin has been completely elucidated within the last five years,[59,60] and major progress has been made in determining the primary amino acid sequence of rhodopsin,[61] the configuration of the molecule,[42,62] and its orientation within the disc membrane[63] (Figure 5).

The molecular components of the photoreceptors are under continuous and vigorous renewal (see Chapter 8, "Retinal Pigment Epithelium") and the

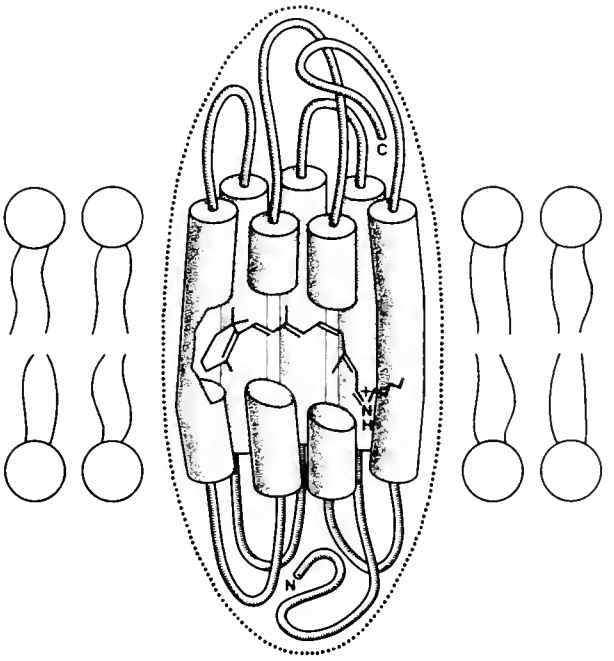


FIGURE 5. A model for the location and structure of rhodopsin in rod cell disk membrane. Rhodopsin is shown as an elongated bundle of 7 helices. Half of rhodopsin's mass is embedded in the lipid bilayer. The rest is distributed equally at each aqueous surface as shown in the diagram by the regions of polypeptide chain connecting the helices. Rhodopsin's chromophore, 11-cis retinal, is shown in a pocket formed by several helices and attached to its lysine-binding site in the carboxyl terminal helix. The carboxyl terminal faces the cytoplasmic surface. The amino acid sequence in rhodopsin has no statistical relationship to the sequence in bacterial rhodopsin. However, the gross structural features of these two different proteins are obviously similar. The most striking difference is the increased length of the aqueous connector chains in rhodopsin, and it is light-induced conformational changes in these connector chains that help activate the light-dependent biochemical changes in the rod cell.

synthesis of glycoproteins and lipids destined for the outer segment is prodigious. The pathway by which membrane proteins are transported from synthesis sites in the rough endoplasmic reticulum and Golgi zone to their assembly site near the base of the connecting cilium has been described. Investigations of the region near the connecting cilium are underway to determine how the photoreceptor controls the distribution of rhodopsin once it is incorporated.[64] Studies of disc development have led to an explicit model for disc morphogenesis in which the disc surfaces and disc rims develop by separate processes of growth from the plasma membrane of the cilium.[65] This model may provide a developmental basis for the finding that a large molecular weight integral membrane protein is confined to the disc rim,[66] whereas rhodopsin is highly mobile and free to roam within the disc membrane.

Some of the many other advances in research on photoreceptors include the following: demonstration that electrically excited K^+ channels participate in the transmission (and modification) of the receptor potential as it travels along the cell toward the synaptic ending,[67] evidence for the presence and possible role of electrically excited Ca^{++} channels in the synaptic ending,[68] and progress in identifying the synaptic transmitter[69,70] and synaptic interactions, including the signal "pooling" most likely mediated by gap junctions that electrically couple receptors of a given class.[71,72]

Finally, a surprising new finding has come from human psychophysical research. Using the Stiles-Crawford effect to detect photoreceptor orientation, investigators have recently found that if the pupil is artifically displaced, the photoreceptors realign to point toward the new position of the displaced pupil. Thus, human receptors appear to be phototropic, actively aligning themselves with their source of light.[73] The mechanism of this phototropism is unknown but may be related to the striking photomechanical movements made by both rods and cones in cold-blooded animals. When inactive—rods during the day, cones during the night—the outer segments appear to protect themselves from light by moving deep into the melanin-containing pigment epithelium. When the photoreceptors are active, the outer segments move to the surface of the retinal pigment epithelium where they can best catch the light.

RESEARCH NEEDS AND OPPORTUNITIES

Photoreceptor Metabolism

Traditionally, most research on photoreceptors has been concerned with structure and physiological functions. However, in the last few years, interest has rapidly increased in research on photoreceptor metabolism, especially the shedding process that results in the renewal of disc outer segment membranes. Concurrently, investigations of the toxicity of light and research on diseases involving photoreceptors have made increasingly obvious the need to know far more about the fundamental metabolic processes in the photoreceptors. Questions that need answers include: What molecules "wear out" or become damaged? How is such molecular wear and tear detected? How are both the size and the composition of a functioning structure like the outer segment maintained while the structure itself is being replaced? What regulatory feedback loops determine the rates of synthesis and shedding? Where is the "clock" that regulates the periodicity of disc shedding? What are the respective roles of cytoplasmic and nuclear factors in metabolic control loops? How are the genes informed of the results of their expression? This brief and incomplete list merely suggests the wide range of questions about normal and abnormal photoreceptor metabolism that can be or are being investigated. Answers to such questions undoubtedly will increase understanding of the mechanisms underlying the most important diseases and degenerations of visual receptors.

A few clues about the wearing out process in the outer segment recently have been discovered in research on amphibian photoreceptors. Single photon responses (quantum bumps) are slower when the light is absorbed in the distal tip,[24] the birefringence of the outer segment varies discontinuously along its length,[74,75] and exposure to light accelerates the rate of outer segment assembly and triggers the shedding process.[76] Many new techniques have become available for pursuing such problems, especially the techniques of cell and tissue culture, and cell separation and sorting. Moreover, powerful new methods for studying, as well as employing, nucleic acids are just beginning to be applied to research on the metabolism of visual receptors. One objective will be to isolate the gene that codes for rhodopsin, determine its nucleotide sequence, establish whether untranslated regions exist within the gene, and examine the relationship between the genes of rhodopsin and cone visual pigments. With the successful isolation of opsin mRNA, for the first time direct genetic studies of visual pigment structure and its genomic organization can be performed.

Phototoxicity

Most rod and cone degenerations almost certainly involve some defect in a metabolic pathway and/or the inability of repair mechanisms to overcome some increasing source of damage. Considerable evidence now suggests that the rate of photoreceptor and macular degenerations can be greatly accelerated by light and that light becomes even more toxic when nutritional deficiencies reduce the effectiveness of metabolic repair and defense mechanisms.

Phototoxic damage is almost always greatest in the central retina,[7,17] perhaps because both the density of photoreceptors and the flux of light falling on the retina are greatest in the region surrounding the optical axis of the eye. The similarities between phototoxic damage to the retina and aging-related maculopathy make further research mandatory on the degree to which light exposure accelerates aging and degeneration in the macula. Indeed, a recent epidemiological study suggests that the prevalence of macular degeneration is lower in people with nuclear lens opacities that filter the light reaching the retina.[77] Additional epidemiological studies are needed to assess the importance of light exposure in influencing the rate at which macular degeneration develops in the aging retina. The influence of occupation and geographic location should also be examined. Aging of the skin is accelerated by exposure to the blue and near-ultraviolet light of the sun,[78] and epidemiological studies suggest that prevalence of cataracts and corneal abnormalities is higher in locations with large amounts of sunlight.[79] It is not known, however, whether the same holds true for macular degenerations of the retina. Are aphakics more susceptible than those with lenses to damage by light from the far blue portion of the spectrum? Is the blue and near-ultraviolet light emitted by arc and fluorescent lights safe for our eyes?[4,7,80]

Nutritional Deficiencies

One of the most likely mechanisms for phototoxicity in the photoreceptors is photooxidation of lipids and other molecules. The photoreceptors contain unusually high concentrations of polyunsaturated phospholipids, which are particularly sensitive to oxidation. Rods and cones also contain high concentrations of vitamin E as well as several other antioxidants.[13,14] Thus, it is not surprising that nutritional deficiencies that deplete these protective agents can produce photoreceptor degeneration and can, in the case of vitamin E, increase the susceptibility of receptors to light toxicity. Diets deficient in vitamins A and E, taurine, selenium, and zinc all lead to photoreceptor degenerations in animals and/or humans.[15,81–84]

Several studies with animals have shown that melanin, especially the melanin of the iris, helps diminish the level of phototoxic damage to the photoreceptors by screening them from some of the ambient light and perhaps by reducing the number of toxic molecules generated by visual pigment chromophores.[85,86] The yellow macular pigment protects the central retina from blue light. This pigment can be depleted in monkeys fed a diet deficient in xanthophyll, and such monkeys soon develop a higher incidence of macular abnormalities.[87]

It is important to know the dietary levels of vitamin E, vitamin A, selenium, zinc, or xanthophyll that will optimally maintain photoreceptors and minimize macular degeneration. In particular, the optimal dietary intake of such micronutrients for older people is unknown. Even though it is likely that optimal intake levels vary widely from one individual to another, there should be some practical way for individuals, other than those already suffering from a recognized nutritional deficiency, to find out whether nutritional supplements are indicated. Optimal micronutrient levels in people with aging-related maculopathy are also unknown. Hence, despite the strongly suggestive studies with animals, little is known about how dietary deficiencies may affect the incidence and progression of aging-related maculopathy.

Photoreceptor Retinal Pigment Epithelium Interactions

The photoreceptors depend vitally on the retinal pigment epithelium: when the retina becomes detached, the photoreceptors may degenerate (see Chapter 6, "Retinal Detachment and Vitreous Disorders" and Chapter 8). Investigators are only beginning to discover the many ways in which the photoreceptors interact with the retinal pigment epithelium. Some information about how vitamin A is stored and transferred back and forth between the photoreceptors and the retinal pigment epithelium has been gained, including some recent advances in characterizing the carrier (binding) proteins for this oil-soluble vitamin.[88,89] Less is known about the exchange of other metabolites and nutrients, such as vitamin E.[90] Nor is much known about how the "indigestible," potentially toxic, debris shed by the photoreceptors affects the aging and/or degeneration of the retinal pigment epithelium cells. Because these cells must engulf so much debris from the photoreceptors, it is entirely possible that some types of retinal pigment epithelium degeneration may be due to the excessive accumulation of toxic materials shed by the photoreceptors, even though the photoreceptors may otherwise appear entirely healthy.

Recently, photoreceptors have been shown to be autophagic (like most other cells), taking in and digesting some of their own components.[91] This information raises questions, such as: Why do the photoreceptors shed their discs? How do the photoreceptors regulate the point of detachment of the distal tips and make the clump of discs they shed "tasty" for retinal pigment epithelial cells while keeping the rest of the outer segment "unpalatable"?

Photoreceptor Physiology

Research on the physiology of photoreceptors is progressing at an excellent pace. In the next few years, the roles of rhodopsin in excitation and adaptation are likely to be elucidated and the role of Ca^{++} determined. In all cells, Ca^{++} and cyclic nucleotides are closely coupled, but the nature of this coupling in photoreceptors is unknown. Numerous enzyme pathways need to be characterized and their role in excitation and adaptation determined. Much more study of ion movements is needed to answer questions, such as: How are Na^+, K^+, Ca^{++}, and other ion channels, exchangers, and pumps distributed throughout the photoreceptor membranes? How do ion movements contribute to signal processing and signal transmission? The location of the ouabain-sensitive Na^+ pump has now been demonstrated with radioautography,[92] and recent evidence suggests that Ca^{++} is pumped out of the outer segment by a Na^+/Ca^{++} exchanger.[57,58] Many more such studies will be needed before the electrophysiology of the photoreceptors is fully understood. Finally, much remains to be learned about signal processing by the synapse. The chemical transmitters employed by photoreceptors must be identified, and the way in which they are synthesized, packaged, released, and inactivated studied. The new techniques for isolating and recording from single cells, and even from small patches of membrane sealed to the tip of the microelectrode, should prove of great value in this effort.

Structure and Dynamics

Despite its apparent structural simplicity, the photoreceptor outer segment is a highly sophisticated and complex structure. Techniques are needed to delineate this structure at the molecular level. The use of monoclonal antibodies seems particularly valuable for locating and identifying the roles of the dozens (or more) structural molecules in the outer segment and cilium. Deep-etch freeze fracture and high voltage EM may greatly improve the spatial resolution of molecular locations. The use of fluorescent labels should aid in detecting some of the key

molecular motions such as the interactions between rhodopsin and the enzymes it activates.

Advances in Raman spectroscopy and peptide sequencing should permit considerable advances in determining the structure of the chromophore site and the way it shifts the absorption spectrum. Moreover, sequencing and labeling techniques should continue to expand knowledge of the structure of rhodopsin. Finally, comparisons between rhodopsin and its bacterial analogs, bacteriorhodopsin and halorhodopsin, may provide valuable insights about both the structure and function of these membrane proteins.

Adaptation Mechanisms

Research on photoreceptors is far ahead of research on other cells in characterizing the physiological roles of adaptation (regulatory) mechanisms. Because different rods and cones have significantly different adaptation characteristics, comparisons between the ion/nucleotide/enzyme systems in rods and cones may reveal general features that will be useful in understanding these systems in other types of cells. Comparison of the kinetics of ion/nucleotide/enzyme systems with the frequency and amplitude of the photoreceptor's light response should help determine which chemical reactions play a direct role in visual excitation.

Animal Models

More extensive morphologic studies must be encouraged to establish the pathological base against which experimental models of human retinal diseases can be appropriately compared. Some of the photoreceptors in these diseased retinas are capable of relatively normal function and structure for many years prior to the clinical onset of visual loss. Altered photoreceptor sensitivity can be demonstrated using improved physiological tests in younger patients who are unaware of visual loss. Parallels with slow-onset neurological disorders, including Huntington's chorea, and various degenerative brain diseases should be considered.

The need for more animal models is obvious considering what information already has been provided by such models, such as the discovery of light toxicity in the rat, an animal far more susceptible to this problem than humans. Deficiencies in phosphodiesterase activity recently have been discovered in two different animals (mouse and dog) with hereditary diseases of the retina,[93,94] and this suggests that such deficiencies may occur in human retinal diseases. Aging undoubtedly plays a role in the development of macular degeneration; and there is a great need for longitudinal studies of human aging to characterize changes in metabolism and suggest ideas for slowing or preventing macular degenerations. The recent advances in techniques for culturing animal tissues and cells should greatly facilitate the development of methods for culturing tissues and cells from diseased human retinas, and lead to progress in understanding the molecular and cellular basis of these human diseases. Short-lived (fast aging) animals may provide models for the study of aging-related changes in retinal processes.

RECOMMENDATIONS

Based on the foregoing assessment of recent accomplishments, current activities, and research needs and opportunities in "Photoreceptors, Visual Pigments, and Phototransduction," the Panel has made the following recommendations concerning research in this subprogram over the next five years. These have been grouped under two headings: Program Base and Program Development Priorities.

The Program Base includes areas of ongoing research where the current level of activity is considered adequate, or areas of ongoing research in which there may be great need for additional activity, but where, in the Panel's judgment, little or no opportunity (new methods or insights) exists at present to justify a significant expansion of effort. Nonetheless, additional applications for research grants in these areas may be funded if they are innovative and of very high quality as determined by the NIH peer review system.

Program Development Priorities include areas of ongoing research in which new knowledge and techniques offer particular opportunities for scientific progress, or promising new areas of research in which there is little or no support at present but where there is both great need and high potential for success. Such areas are judged to warrant significantly increased support over the next five years, provided that high quality applications for research grants in these areas are forthcoming.

Program Base

- Investigate the photochemical events of visual excitation by chemical, biochemical, and biophysical studies of the visual pigments.
- Characterize the macromolecular constituents of photoreceptor membrane systems to establish the orientation and interactions of rhodopsin and other key protein and lipid components and to delineate the role of the membrane microenvironment in modulating physiologic function.
- Investigate the phototransduction process, emphasizing research that characterizes the perti-

nent biochemical and metabolic events at the molecular level.

- Characterize the adaptation mechanisms that regulate the photoreceptor threshold, speed of response, and dynamic range, stressing investigations at the molecular level. Compare the ion/enzyme/nucleotide systems in photoreceptors with different adaptation characteristics.
- Investigate the role and mechanisms of synaptic interactions between photoreceptors and the cells they innervate (see Chapter 10, "Retinal Organization, Neurotransmission, and Adaptation").

Program Development Priorities

- Investigate photoreceptor metabolism, its control, and metabolic interactions with the retinal pigment epithelium, particularly vitamin A and nucleotide metabolism, membrane biosynthesis and degradation, and protein phosphorylation (see Chapter 8).
- Investigate environmental (for example, light exposure), nutritional (for example, vitamin intake), and other factors that may affect the quality of photoreceptor function and the rate of photoreceptor degeneration and aging (see Chapters 4, 5, and 8).
- Use existing animal models of human photoreceptor degenerative disorders and develop new models in systematic attempts to prevent or slow the degenerative processes (see Chapters 4 and 5; and Chapter 14, "Tissue Acquisition and Distribution: Human Donor Eyes and Animal Models").

RESOURCE REQUIREMENTS

After reviewing current research grant support in each of these categories and assessing the need and potential for future development, the Panel has estimated the number of projects it believes are needed to carry out its recommendations in FY 1983. These estimates are shown in the table on the following page. For a discussion of the general basis and significance of these projections, see the "Summary" at the beginning of this report.

RESOURCE TABLE

PHOTORECEPTORS, VISUAL PIGMENTS, AND PHOTOTRANSDUCTION

	No. of Grants FY 1981	Panel Recommendation FY 83	
		Add. Grants	Total Grants
Program Base			
A. Investigate the photochemical events of visual excitation by means of chemical, biochemical, and biophysical studies.	23	−2	21
B. Characterize photoreceptor membrane systems.	21	−1	20
C. Investigate the phototransduction process.	31	−3	28
D. Characterize the adaptation mechanisms that regulate the photoreceptor threshold, speed of response, and dynamic range.	8	0	8
E. Investigate the role and mechanism of synaptic interactions between photoreceptors and the cells they innervate.	7	0	7
Program Development Priorities			
A. Investigate photoreceptor metabolism, its control, and metabolic interactions with the RPE.	18	3	21
B. Investigate environmental, nutritional, and other factors that may affect the quality of photoreceptor function, degeneration, and aging.	4	3	7
C. Use animal models in attempts to prevent or slow down photoreceptor degenerative processes.	*	[2]*	*
Subtotal Grants	**112**	**0**	**112**
(% of Program)	**(29)**	**(0)**	**(23)**
Total Estimated Cost	**$9,514,000**	**$2,246,000**	**$11,760,000**

*Counted in Developmental and Hereditary Disorders subprogram. See Chapter 4.

REFERENCES

1. Noell WK, Walker VS, Kang BS, et al: Retinal damage by light in rats. *Invest Ophthalmol Vis Sci* 5:450–473, 1966.
2. Lanum J: The damaging effects of light on the retina. *Surv Ophthalmol* 22:221–249, 1978.
3. Williams TP, Baker BN (eds): *The Effects of Constant Light on Visual Processes*. New York, Plenum Press, 1980.
4. Proceedings of a Symposium on Intense Light Hazards in Ophthalmic Diagnosis and Treatment. *Vision Res* 12:1033–1203, 1980.
5. Ham WT Jr, Ruffolo JJ Jr, Mueller HA, et al: The nature of retinal radiation damage: Dependence on wavelength, power level, and exposure time. *Vision Res* 20:1105–1111, 1980.
6. Sperling HG, Johnson C, Harwerth RS: Differential spectral photic damage to primate cones. *Vision Res* 20:1117–1125, 1980.
7. Sykes SM, Robison WG Jr, Waxler M, et al: Damage to the monkey retina by broad-spectrum fluorescent light. *Invest Ophthalmol Vis Sci* 20:425–434, 1981.
8. Henry MM, Henry LM, Henry LM: A possible cause of chronic cystic maculopathy. *Ann Ophthalmol* 9:455–457, 1977.
9. Tso MO, Fine BS, Zimmerman LE: Photopic maculopathy produced by the indirect ophthalmoscope: I. Clinical and histopathologic study. *Am J Ophthalmol* 73:689–699, 1972.
10. Kuwabara T, Gorn RA: Retinal damage by visible light. *Arch Ophthalmol* 79:69, 1968.
11. Calkins JL, Hochheimer BS: Retinal light exposure from operation microscopes. *Arch Ophthalmol* 97:2363–2367, 1979.
12. Dilley RA, McConnell DG: Alpha-tocopherol in the retinal outer segments of bovine eyes. *J Membr Biol* 2:317–323, 1970.
13. Farnsworth CC, Dratz EA: Oxidative damage of retinal rod outer segment membranes and the role of vitamin E. *Biochim Biophys Acta* 433:556–561, 1976.
14. Shvedova AA, Sidorov AS, Novikov KN, et al: Lipid peroxidation and electric activity of the retina. *Vision Res* 19:49–55, 1979.
15. Hayes KC: Retinal degeneration in monkeys induced by deficiencies of vitamin E or A. *Invest Ophthalmol Vis Sci* 13:499–510, 1974.
16. Robison WG Jr, Kuwabara T, Bieri JG: Vitamin E deficiency and the retina: Photoreceptor and pigment epithelial changes. *Invest Ophthalmol Vis Sci* 18:683–690, 1979.
17. Young RW: A theory of central retinal disease, in Sears ML: *New Directions in Ophthalmic Research*. New Haven and London, Yale University Press, 1981, pp 237–270.
18. Besharse C, Terrill RO, Dunis DA: Light-evoked disc shedding by rod photoreceptors in vitro: Relationship to medium bicarbonate concentration. *Invest Ophthalmol Vis Sci* 19:1512–1517, 1980.
19. Hall MO: Phagocytosis of light- and dark-adapted rod outer segments by cultured pigment epithelium. *Science* 202:526–528, 1978.
20. Goldman AI, Teirstein RS, O'Brien PJ: The role of ambient lighting in circadian disc shedding in the outer segment of the rat retina. *Invest Ophthalmol Vis Sci* 19:11, 1980.
21. Miller WH: Molecular mechanisms of photoreceptor transduction. *Curr Top Membr Transport* 15, 1981.
22. Yau KW, Lamb TD, Baylor DA: Light-induced fluctuations in membrane current of single toad rod outer segments. *Nature* 269:78–80, 1977.
23. McBurney RN, Normann RA: Current and voltage responses from single rods in toad retina. *J Gen Physiol* 70:12a, 1977.
24. Baylor DA, Lamb TD, Yau KW: The membrane current of single rod outer segments. *J Physiol (Lond)* 288:589–611, 1979.
25. Baylor DA, Lamb TD, Yau KW: Responses of retinal rods to single photons. *J Physiol (Lond)* 288:613–634, 1979.
26. Hagins WA, Penn RD, Yoshikami S: Dark current and photocurrent in retinal rods. *Biophys J* 10:380–412, 1970.
27. Korenbrot JI, Cone RA: Dark ionic flux and the effects of light in isolated rod outer segments. *J Gen Physiol* 60:20–45, 1972.
28. Barlow HB: Dark and light adaptation: Psychophysics, in Jameson D, Hurvich LM (eds): *Handbook of Sensory Physiology*. New York, Springer-Verlag, 1972, vol 7, pt 4, pp 1–28.
29. Barlow HB: Retinal and central factors in human vision limited by noise, in Barlow HB, Fatt P: *Vertebrate Photoreception*. London, Academic Press, 1977, pp 337–351.
30. Yau KW, Matthews G, Baylor DA: Thermal activation of the visual transduction mechanism in retinal rods. *Nature* 279:785–786, 1979.
31. Baylor DA, Matthews G, Yau KW: Two components of electrical dark noise in toad retinal rod outer segments. *J Physiol (Lond)* 309:591–621, 1980.
32. Honig B, Ebrey T, Callender RH, et al: Photoisomerization, energy storage, and charge separation: A model for light energy transduction in visual pigments and bacteriorhodopsin. *Proc Natl Acad Sci USA* 76:2503–2507, 1979.
33. Honig B, Dinur U, Nakanishi K, et al: An external point-charge model for wavelength regulation in visual pigments. *J Am Chem Soc* 101:7084–7086, 1979.
34. Hubbard R, Kropf A: The action of light on rhodopsin. *Proc Natl Acad Sci USA* 44:130, 1958.
35. Eyring G, Curry B, Mathies R, et al: Interpretation of the resonance spectrum of bathorhodopsin based on visual pigment analogues. *Biochemistry* 19:2410–2418, 1980.
36. Cooper A: Energy uptake in the first step of visual excitation. *Nature* 282:531–533, 1979.
37. Doukas AG, Aton B, Callender RH, et al: Resonance raman studies of bovine metarhodopsin I and metarhodopsin II. *Biochemistry* 17:2430–2435, 1978.
38. Kuhn H, Mommertz O, Hargrave PA: Evidence for a light-dependent conformational change at rhodopsin's surface. *FEBS Lett*, to be published.
39. Bennett N: Light-induced interactions between rhodopsin and the GTP-binding protein. *Eur J Biochem* 123:133, 1982.

40. Rafferty CN: Light-induced perturbation of aromatic residues in bovine rhodopsin and bacteriorhodopsin. *Photochem Photobiol* 29:109–120, 1979.
41. Osborne HB, Nabedryk-Viala E: The conformation of membrane-bound and detergent-solubilized bovine rhodopsin. *Eur J Biochem* 89:81–88, 1978.
42. Chabre M: Diamagnetic anistotropy and orientation of alpha-helix in frog rhodopsin and meta II intermediate. *Proc Natl Acad Sci USA* 75:5471–5474, 1978.
43. Godchaux W, Zimmerman WF: Membrane dependent guanine nucleotide binding and GTPase activities of soluble proteins from bovine rod cell outer segments. *J Biol Chem* 254:7874, 1979.
44. Fung BK, Stryer L: Photolyzed rhodopsin catalyzes the exchange of GTP for bound GDP in retinal rod outer segments. *Proc Natl Acad Sci USA* 77:2500–2504, 1980.
45. Kuhn H: Light- and GTP-regulated interaction of GTPase and other proteins with bovine photoreceptor membranes. *Nature* 283:587–589, 1980.
46. Miki N, Keirns JJ, Marcus FP, et al: Regulation of cyclic nucleotide concentrations in photoreceptors: An ATP-dependent stimulation of cyclic nucleotide phosphodiesterase by light. *Proc Natl Acad Sci USA* 70:3820–3824, 1973.
47. Yee R, Liebman PA: Light-activated phosphodiesterase of the rod outer segment. *J Biol Chem* 253:8902–8909, 1978.
48. Bownds MD, Dawes J, Miller J, et al: Phosphorylation of frog photoreceptor membranes induced by light. *Nature* 237:125–127, 1972.
49. McDowell JH, Kuhn H: Light-induced phosphorylation of rhodopsin in cattle photoreceptor membranes: Substrate activation and inactivation. *Biochemistry* 16:4054–4060, 1977.
50. Kilbride P, Ebrey TG: Light-initiated changes of cyclic guanosine monophosphate levels in the frog retina measured with quick-freezing techniques. *J Gen Physiol* 74:415–426, 1979.
51. Szuts EZ: Calcium flux across disk membranes: Studies with intact rod photoreceptors and purified disks. *J Gen Physiol* 76:253–286, 1980.
52. Gold GH, Korenbrot JI: Light-induced calcium release by intact retinal rods. *Proc Natl Acad Sci USA* 77:5557–5561, 1980.
53. Yoshikami S, George JS, Hagins WA: Light-induced calcium fluxes from outer segment layer of vertebrate retinas. *Nature* 286:395–398, 1980.
54. Yoshikami S, Hagins WA: Control of the dark current in vertebrate rods and cones, in Langer H (ed): *Biochemistry and Physiology of Visual Pigments*. New York, Springer-Verlag, 1973, pp 245–255.
55. Brown JE, Coles JA, Pinto L: Effects of injection of calcium and EGTA into outer segments of retinal rods of *Bufo marinus*. *J Physiol (Lond)* 269:707–722, 1977.
56. Hagins WA, Yoshikami S: Intracellular transmission of visual excitation in vertebral photoreceptors: Electrical effects of chelating agents introduced into rods by vesicle fusion, in Barlow HB, Fatt P (eds): *Vertebrate Photoreception*. New York, Academic Press, 1977, pp 97–139.
57. Schnetkamp PPM: Ion selectivity of the cation transport system of isolated intact cattle rod outer segments. *Biochim Biophys Acta* 598:66–71, 1980.
58. Yoshikami S, Hagins WA: Sodium-calcium exchange and the kinetics of the rod response. *Biophys J* 33(pt 2):288a, 1981.
59. Fukuda M, Papermaster D, Hargrave P: Rhodopsin carbohydrate: Structure of small oligosaccharides attached at two sites near the NH^2 terminus. *J Biol Chem* 254:8201–8207, 1979.
60. Liang C, Yamashita K, Muellenberg C, et al: Structure of the carbohydrate moieties of bovine rhodopsin. *J Biol Chem* 254:6414–6418, 1979.
61. Hargrave P, Fong S-L, McDowell J, et al: The partial primary structure of bovine rhodopsin and its topography in the retinal rod cell disc membrane. *Neurochem* 1:231–244, 1980.
62. Albert A, Litman B: Independent structural domains in the membrane protein bovine rhodopsin. *Biochemistry* 17:3893–3899, 1978.
63. Hargrave P: The amino-terminal tryptic peptide of bovine rhodopsin: A glycopeptide containing two sites of oligosaccharide attachment. *Biochim Biophys Acta* 492:83–94, 1977.
64. Peters K, Schneider B, Papermaster D: Ultrahigh resolution scanning electron microscopy of a periciliary ridge complex of frog retinal rod cells. *J Cell Biol* 91:273a, 1981.
65. Anderson DH, Fisher SK, Steinberg RH: Mammalian cones: Disc shedding, phagocytosis, and renewal. *Invest Ophthalmol Vis Sci* 17:117–133, 1978.
66. Papermaster DS, Converse CA, Zorn MA: Biosynthetic and immunochemical characterization of a large protein in frog and cattle rod outer segment membranes. *Exp Eye Res* 23:105–116, 1976.
67. Werblin FS: Light, voltage, and time-dependent components of the rod response. *Sens Processes* 2:306, 1978.
68. Fain GL, Gerschenfeld HM, Quandt FN: Calcium spikes in toad rods. *J Physiol (Lond)* 303:495, 1980.
69. Lam DMK: Biosynthesis of acetylcholine in turtle photoreceptors. *Proc Natl Acad Sci USA* 69:1987–1991, 1972.
70. Wu SM, Dowling JE: L-aspartate: Evidence for a role in cone photoreceptor synaptic transmission in the carp retina. *Proc Natl Acad Sci USA* 75:5205–5209, 1978.
71. Raviola E, Gilula NB: Gap junctions between photoreceptor cells in the vertebrate retina. *Proc Natl Acad Sci USA* 70:1677–1681, 1973.
72. Fain GL, Gold GH, Dowling JE: Receptor coupling in the toad retina. *Cold Spring Harbor Symp Quant Biol* 40:547–561, 1976.
73. Enoch JM, Birch DG: Evidence for alteration in photoreceptor orientation. *Ophthalmology* 87:821–833, 1980.
74. Kaplan MW: Birefringence bands in frog rod outer segments depend on light/dark cycles. *Invest Ophthalmol Vis Sci* 19(suppl):160, 1980.
75. Andrews LD, Mackenzie JM Jr, Basinger SF: Birefringent periodicities in amphibian rod outer segments. *Invest Ophthalmol Vis Sci* 20(suppl):151, 1981.

76. LaVail MM: Rod outer segment disk shedding in rat retina: Relationship to cyclic lighting. *Science* 194:1071, 1976.
77. Sperduto RD, Hiller R, Seigel D: Lens opacities and senile maculopathy. *Arch Ophthalmol* 99:1004–1008, 1981.
78. Rook A, Wilkinson DS, Ebling FJG (eds): *Textbook of Dermatology*. Oxford, Blackwell Scientific Publications, 1979.
79. Lerman S: *Radiant Energy and the Eye*. New York, Macmillan Publishing Co, Inc, 1980, pp 125, 162–163.
80. Calkins JL, Hochheimer BF: Retinal light exposure from ophthalmoscopes, slit lamps, and overhead surgical lamps: An analysis of potential hazards. *Invest Ophthalmol Vis Sci* 19:1009–1015, 1980.
81. Robison WG Jr, Kuwabara T, Bier JG: Vitamin E deficiency and the retina: Photoreceptor and pigment epithelial changes. *Invest Ophthalmol Vis Sci* 18:683–690, 1979.
82. Schmidt SY, Berson EL, Watson G, et al: Retinal degeneration in cats fed casein: III. Taurine deficiency and ERG amplitudes. *Invest Ophthalmol Vis Sci* 16:673–678, 1977.
83. Farnsworth CC, Stone WL, Dratz EA: Effects of vitamin E and selenium deficiency on the fatty acid composition of rat retinal tissue. *Biochim Biophys Acta* 552:281–293, 1979.
84. Campbell IA, Elmes PC: Ethambutal and the eye: Zinc and copper. *Lancet* 7934:711, 1975.
85. Rapp LM, Williams TP: A parametric study of retinal light damage in albino and pigmented rats, in Williams TP, Baker BN (eds): *The Effects of Constant Light on Visual Processes*. New York, Plenum Press, 1980, pp 135–159.
86. Williams TP, Rapp LM: The role of ocular pigmentation in protecting against retinal light damage. *Vision Res* 20:1127–1131, 1980.
87. Malinow MR, Feeney-Burns L, Peterson LH, et al: Diet-related macular anomalies in monkeys. *Invest Ophthalmol Vis Sci* 19:857–863, 1980.
88. Heller J, Bok D: A specific receptor for retinal binding protein as detected by the binding of human and bovine retinol binding protein to pigment epithelial cells. *Am J Ophthalmol* 81:93–97, 1976.
89. Saari JC, Futterman S, Bredberg L: Cellular retinol- and retinoic acid-binding proteins of bovine retina. *J Biol Chem* 253:6432, 1978.
90. Fletcher RT, Chader GJ: A vitamin E binding proteint of the pigment epithelium. *Invest Ophthalmol Vis Sci* 20(suppl):209, 1981.
91. Reme CE, Knop M: Autophagy in frog visual cells in vitro. *Invest Ophthalmol Vis Sci* 19:439–456, 1980.
92. Stirling CE, Lee A: ^{3}H-ouabain autoradiography of frog retina. *J Cell Biol* 85:313–324, 1980.
93. Farber DB, Lolley RN: Enzymatic basis for cyclic GMP accumulation in degenerative photoreceptor cells of mouse retina. *J Cyclic Nucleotide Res* 2:139, 1976.
94. Aguirre G, Farber D, Lolley R, et al: Rod-cone dysplasia in Irish setters: A defect in cyclic GMP metabolism in visual cells. *Science* 201:1133–1134, 1978.

10

RETINAL ORGANIZATION, NEUROTRANSMISSION, AND ADAPTATION

INTRODUCTION

THE RETINA IS far more than an array of photoreceptors. In addition to rods and cones, it contains at least five types of other nerve cells (neurons), which begin the processing of visual information.[1] A substantial amount of neural processing occurs within the retina; in fact, the basic response properties of neurons throughout the visual system, responding to the onset and/or cessation of illumination depending on the nature and position of the light stimuli, is established within the retina.[2]

The retina consists of three cellular layers. The distal-most (outer nuclear) layer contains the cell bodies (perikarya) of the photoreceptors; the middle (inner nuclear) layer consists of the perikarya of both second-order neurons (bipolar and horizontal cells) and third-order neurons (amacrine and interplexiform cells); and the innermost cellular layer contains the perikarya of the third-order ganglion cells, whose axons run along the surface of the retina to the optic disc where they leave the eye as a bundle within the optic nerve.

Between the cellular layers of the retina are two synaptic or plexiform layers, where the processes of the various retinal cells make junctions, or synapses, with one another. Within each plexiform layer, the processes of four cell types can interact. For example, in the outer plexiform layer there are processes of photoreceptor, bipolar, horizontal, and interplexiform cells, whereas the inner plexiform layer contains the processes of bipolar, amacrine, interplexiform, and ganglion cells. All visual information passes from one set of neurons to another at least twice within the retina. This takes place exclusively within the two plexiform layers of the retina, where information processing occurs by means of the complex synaptic interactions of the various retinal cells with one another. The results of this processing appear ultimately in the message carried by the ganglion cell axons (the optic nerve) to the brain.

Research over the past three decades, employing extracellular recording techniques has succeeded in describing the receptive field properties and response characteristics of ganglion cells from a wide variety of species. These studies have shown that two basic types of processing occur within the plexiform layers of the retina. One class of ganglion cell shows evidence of *spatial* processing; the response of the cell depends on the position of the stimulus on the photoreceptor mosaic.[3–5] That is, depending on where a spot of light is positioned on the retina, the cell will respond vigorously either at the onset of light or at its cessation. Another class of ganglion cell shows evidence of *temporal* processing.[5–9] These cells respond only weakly to spots of light projected onto the retina. However, if the spot of light is moved across the retina, the cells respond very vigorously. Thus, these cells are concerned less with the position of illumination on the retina than with its temporal properties. Some cells of this class show even more sophisticated kinds of processing; they respond preferentially to spots of light moving in one direction on the retina;[7–9] if the spot is moved in the opposite direction, the cell responds little if at all.

The complexity of the ganglion cells' response properties underscores the fact that the retina is a part of the brain, displaced into the eye during an early stage of embryological development. Thus study of the retina also provides insights concerning

brain function. Indeed, the retina is viewed by many as a model piece of the brain and many brain researchers are turning to the retina because of its many advantages for study. For example, the retina is the most accessible part of the brain; it can be easily stimulated with light projected on the receptors; its output by way of the optic nerve can be readily monitored; and, finally, the retina can be removed from the eye and maintained functionally in an artificial environment for long periods of time.

Earlier in this report, numerous diseases of the retina are described, but little is known about their underlying causes. Clearly, a detailed understanding of retinal function is required if the nature and significance of retinal lesions are to be understood and if effective therapy is to be developed.

SUBPROGRAM OBJECTIVES

- To define the anatomical, biochemical, physiological, and pharmacological principles underlying the functional organization and adaptative properties of the retina.
- To determine the metabolic processes and physiological conditions necessary for the maintenance and proper functioning of the retina.
- To define the principles of retinal development, including the mechanism of retinal induction, differentiation of various retinal cell types, synaptogenesis, onset of retinal function, and retinal genetics.
- To identify the cellular origins of clinically relevant electrical potentials and develop noninvasive methods for studying retinal function, understand retinal disease processes, and develop rational therapies for retinal disorders.

OVERVIEW OF CURRENT RESEARCH SUPPORT

Research on the retina has attracted some of the ablest research workers in the vision research field. Thus, substantial numbers of excellent research proposals have been submitted to the National Institutes of Health over the past few years, and a high percentage of these proposals have been approved and funded. For example, over the past four years an average of 32 proposals per year in the area of retinal organization and visual adaptation have been submitted, and, on average, 29 have been approved and 20 funded.

The National Eye Institute supports virtually all the basic retinal research in this country. In FY 1981 the NEI funded 64 research grants in this area at a total cost of $4,729,000. The National Science Foundation provides some marginal support; limited funding is derived also from some private eye foundations, such as Fight for Sight, Inc., and the National Retinitis Pigmentosa Foundation. Salary support for a few of the young investigators in the field has come from other private foundations, such as the Alfred P. Sloan Foundation.

It is clear that basic retinal research has been well-supported over the past several years and that this support has been rewarded by much excellent research and remarkable progress. This, in turn, has encouraged further research and additional workers, especially able younger researchers, to enter the field. Thus, there is a strong ongoing effort, and a justifiable need for continued funding exists. However, certain worthwhile projects remain unsupported, emphasizing the fact that additional funds could profitably be used in this research area.

RECENT ACCOMPLISHMENTS

Over the past 15 years, an enormous amount of information has been gained about intraretinal neuronal mechanisms through anatomical, physiological, and biochemical studies. The retina is recognized as one of the best analyzed parts of the brain, and how it is functionally organized is understood in broad outline. For example, it is known that through the interactions of receptors, horizontal, and bipolar cells in the outer plexiform layer of the retina, a spatial type of analysis is performed on visual information which is reflected in the responses of one of the classes of ganglion cells described above. In the inner plexiform layer, the temporal properties of the light stimuli are sorted, primarily by the amacrine cells, and accentuated by the interactions of amacrine cells with bipolar and ganglion cells. The temporal aspects of illumination are reflected in the responses of the second basic class of ganglion cell, also described above. Furthermore, there is evidence that the different classes of ganglion cells in some species project to different parts of the brain.[9] Thus, in the retina as well as in central visual structures, both serial and parallel processing of visual information occurs.

Much of the progress in understanding the functional organization of the retina has resulted from the application to the retina of two techniques

introduced within the past two decades, electron microscopy and intracellular recording. Electron microscopy has provided the resolution to visualize the synapses between neurons, and so has made it possible to begin elucidating the "wiring" of the retina.[10–15] The combination of standard electon microscopic techniques with more recently discovered specialized methods has enabled researchers to describe in greater detail the connections between the retinal neurons.

Employing such techniques, it has become possible recently to determine the precise synaptic connections of cells by using specific neurotransmitter substances.[16,17] Figure 1 is a diagram illustrating in schematic fashion the five kinds of neurons found in the retina—the horizontal (H), bipolar (B), amacrine (A), interplexiform (IP), and ganglion (G) cells—the synaptic junctions they make with the receptor terminals and with each other as revealed by electron microscopy, and finally, something of the organization of the synaptic contacts in the plexiform layers. This figure originally was pre-

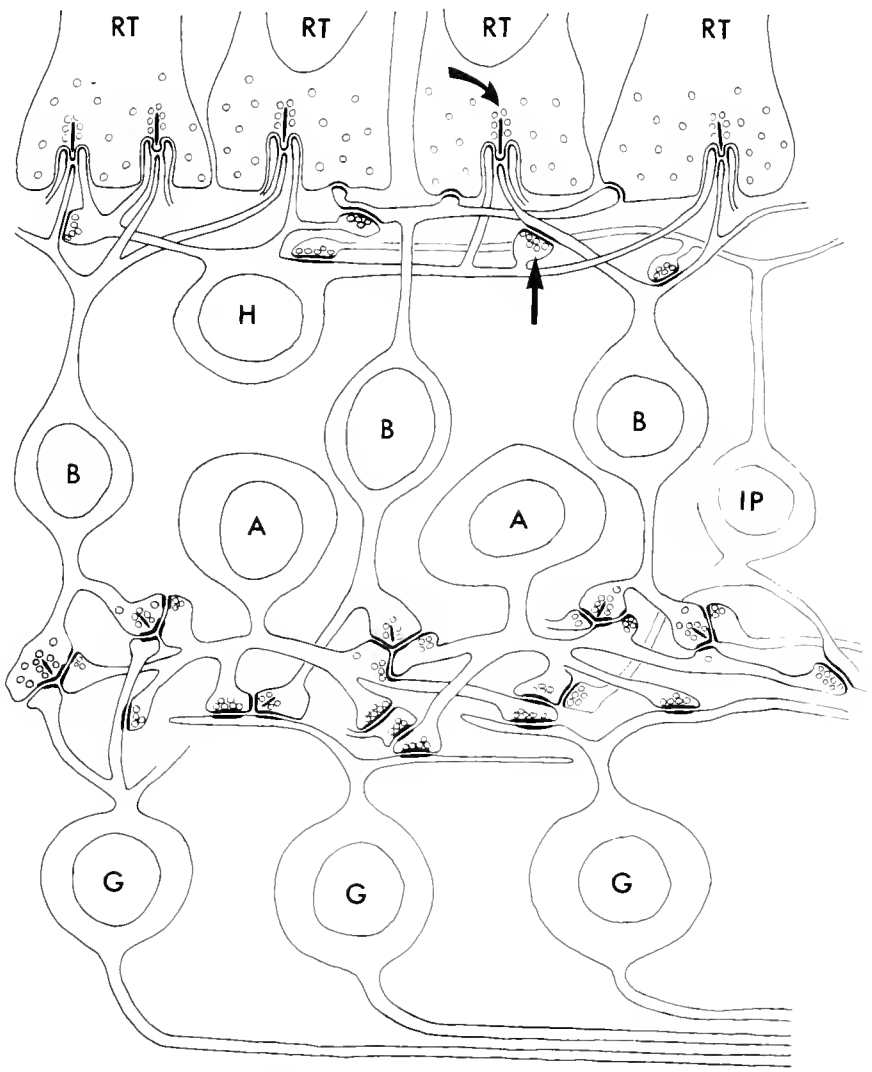


FIGURE 1. Schematic showing synaptic connections in a vertebrate retina: horizontal = H, bipolar = B, amacrine = A, interplexiform = IP, and ganglion = G cells.

pared to show synaptic contacts in the frog retina;[18] it now appears that many of these observations can be generalized to other retinas including that of man.

In all retinas so far studied, there are two major types of synapses, which are believed to function in a chemical fashion. That is, intercellular communication is mediated by a neurotransmitter substance that is released by the presynaptic process and diffuses across the synaptic cleft to interact with specialized receptor sites on the membrane of the postsynaptic cell; this interaction leads either to excitation or inhibition of the postsynaptic neuron. The first of these chemical junctions is termed a ribbon synapse and is characterized by an electron-dense ribbon or bar in the presynaptic element (curved arrow, Figure 1). Ribbon synapses are made in the retina exclusively by the receptor and bipolar cells.[11,19-21] The second kind of chemical synapse observed in the retina is termed a conventional synapse and is characterized by a cluster of synaptic vesicles that are found in the presynaptic terminal close to the presumed synaptic site (straight arrow, Figure 1). These synapses are made by the horizontal, amacrine, and interplexiform cells.[22-24]

Some specialized junctions are also observed in the retina that are believed to mediate direct electrical interactions between the neurons. Such electrical synapses have been observed between photoreceptors, between horizontal cells, between amacrine cells, and between certain bipolar and amacrine cells.[13,25,26] As yet, investigators understand little of the functional significance of the electrical synapses found in the retina.

The horizontal and amacrine cells have aroused particular interest among investigators studying retinal and neuronal organization. These neurons have long been thought to play key roles in the integration of information within the plexiform layers of the retina, and recent anatomical and physiological experiments clearly confirm this idea.[27,28] Many horizontal cells and probably all amacrine cells do not have anatomically identifiable axons.[1,14] The processes of these axonless cells appear alike when viewed by light microscopy; electron microscopy shows that the same process may be both pre- and postsynaptic (see Figure 1). The fact that single processes of horizontal and amacrine cells may both make and receive synapses suggests the possibility of very local interactions occurring between the processes of these neurons and the other retinal elements. The significance of these interactions is not well understood. However, the horizontal and amacrine cells of the retina may provide a model for similar axonless or short-axon cells that occur elsewhere in the brain.

One of the most interesting recent advances in understanding the retinal organization has been the recognition of interplexiform cells (cell labeled IP in Figure 1). The existence of these neurons was not appreciated until just over a decade ago, when fluorescence microscopy unequivocally demonstrated their presence in certain retinas.[16] They have since been observed in a number of species, and are now believed to be present in all retinas. Electron microscopy has shown that these cells receive their synaptic input in the inner plexiform layer and that they make abundant synapses in the outer plexiform layer[16,24] (Figure 1). The interplexiform cell thus appears to provide a centrifugal pathway for information flow in the retina, from the inner to the outer plexiform layers. The functional role of these neurons is currently being investigated actively, and some recent results suggest that they may modify the activity of certain of the horizontal, bipolar, and amacrine cells.

Figure 1 indicates another aspect of retinal synaptic organization that has been culled from comparative anatomical studies of the retina, namely, that some retinal ganglion cells appear to receive substantial input directly from bipolar cells (left side, Figure 1), whereas other ganglion cells receive little or no direct input from the bipolar cells (middle and right side of Figure 1). The latter ganglion cells derive most or all of their input from amacrine cell processes. Research has indicated that ganglion cells that receive the bulk of their input from the amacrine cells show particular sensitivity to moving stimuli.[18,29,30] Recent work in the cat retina has shown further that rod information is transmitted to ganglion cells exclusively by way of amacrine cell processes.[25]

Another important aspect of the anatomical organization of the retina demonstrated recently[31,32] is a functional sublayering of the inner plexiform layer. Cells that respond to light primarily with depolarizing ("on") responses extend their processes in the lower part of the inner plexiform layer (sublamina a), whereas cells that respond primarily with hyperpolarizing ("off") responses extend their processes in the upper part of the layer (sublamina b).

The second technique that has revolutionized understanding of retinal mechanisms is intracellular recording, which has permitted exploration of the physiological properties of individual retinal neurons. Thus, investigators now understand the basic response properties of all the major classes of retinal neurons, and are beginning to understand how the responses of the neurons proximal to the receptors are formed by the synaptic interactions between the various neurons.[33-38] Much of this research has been conducted in cold-blooded vertebrates, such as turtles, salamanders, or fish, primarily because the cells in these animals are larger and the retinas hardier than those of mammals. However, results of intracellular recordings that are beginning to be made in mammals suggest that the basic response properties of retinal neurons are similar in both the

cold- and warm-blooded animals.[39,40] Future progress in understanding the physiology of retinal neurons will require studies employing both kinds of animals.

One of the most interesting and unexpected findings obtained with intracellular recording was that the distal retinal cells—the receptors, horizontal, and bipolar cells—respond to light with sustained graded potentials.[33–35,41–44] Action potentials are never seen in association with these potentials. The distal retinal cells are the first neurons in the vertebrate brain that have been shown to function in this way. Furthermore, most of these distal retinal potentials are hyperpolarizing in sign; this observation has led to the realization that the distal part of vertebrate retina behaves physiologically as though darkness maximally stimulates the system and illumination turns the system off.[45–48] Why this is so remains a mystery but someday may help to explain why cells of the distal retina, especially the photoreceptors, are so susceptible to damage by a number of factors including prolonged illumination, a variety of toxic substances, and inherited gene defects.

Intracellular recordings from the distal retinal neurons have also shown that certain of the horizontal cells are inhibitory interneurons, antagonizing the light responses of certain receptors and the bipolar cells.[27,34,36,49] Because horizontal cells extend their processes over a much wider area of the retina than do bipolar or receptor cells, an antagonistic center-surround receptive field organization is observed in some receptors and in all the bipolar cells recorded from so far. The ganglion cells in the retina that show center-surround receptive field antagonism appear to be those that receive a substantial input directly from the bipolar cells[50] (left side of Figure 1).

Intracellular recordings have also shed light on the function of the more proximal retinal neurons and on the processing occurring in the inner plexiform layer. For example, many, but not all, amacrine cells respond to spots of light projected onto the retina with transient, depolarizing potentials at both the onset and cessation of illumination.[34,35,51] These "on/off" neurons are particularly well-suited to signal the presence of moving stimuli, since their activity rapidly dies away in the presence of steady illumination.[52] Such transiently responding amacrine cells appear to provide a substantial input to those ganglion cells that respond preferentially to movement, and their interactions with each other and with the ganglion cells appear responsible for the complex properties that some ganglion cells display, including directional selectivity.[28,53] The right hand side of Figure 1 shows the kind of complex synaptic circuitry in the inner plexiform layer, involving serial and reciprocal amacrine cell synapses, that might underly such responses.

Research on the membrane properties of individual retinal neurons and the kinetics of synaptic transmission within the retinal network has benefited from the application of methods borrowed from physics and engineering. These include studies of individual membrane channels, membrane noise, voltage-current curves, and network analysis.

For example, the spontaneous release of synaptic transmitter by nerve cells results in a random, continuous fluctuation in the permeability to certain ions in the postsynaptic membrane. Studies of the relation of this fluctuation to the voltage level of the presynaptic cell[54] or the external application of transmitter substance[55] have yielded useful information about the dynamics of transmitter release and provide a means of testing neurotransmitter candidates. Another important application of this form of "noise analysis" is in determining the absolute threshold of retinal cells[56,57] and, indeed, assessing the performance of the entire visual system near threshold.[58]

The activation of nerve cells depends on not only the kinetics of transmitter release by the presynaptic element but also the number and nature of specific ion channels in the postsynaptic membrane, some of which are affected by transmitters, and others by voltage. The properties of these channels can be studied by noting the changes in membrane voltage induced by injection of a current pulse of known magnitude and polarity.[59,60] The shape of the resulting voltage/current curve, in combination with manipulation of the ion composition of the bathing medium[61] and the use of specific pharmacological ion channel blockers,[62] provides insight into the number and nature of the ionic channels in the membranes of the retinal neurons. These, in turn, dictate the cellular responses that underlie the distinct light-responsive properties of diverse retinal microcircuits.

In addition to physiological measures of synaptic function, great insight has been gained, through the application of new methods, into the molecular organization of retinal synapses. These include rapid freezing,[63] deep etching,[64] immunocytochemical labeling of membrane proteins,[65] and lectin binding of membrane sugar residues.[66] Collectively, these techniques permit dissection of the structural components of the synaptic membrane[67] into transmitter receptor sites and their associated ion channels, paramembranous proteins, and structural support elements. Most importantly, they pave the way for studies of synaptic development, renewal, and pathology at a level impossible to conceive only a decade ago.

The technological advances in studying synaptic processes have spurred a concerted effort by a number of workers to understand the mechanisms underlying the synaptic interactions occurring within the plexiform layers of the retina. As a result,

neurotransmitters that carry the signal from one neuron to another across the synapse have been identified. Surprisingly, the retina and other parts of the brain appear to employ a relatively large number of such neurotransmitter substances for reasons that remain obscure. Substantial evidence exists for at least a dozen different substances serving as potential neurotransmitters in the retina, but the transmitters for about one-half the retinal neurons are still not known.

For example, in the outer plexiform layer, it has been shown that the acidic amino acids affect second-order cells in a manner that closely mimics the naturally-occurring receptor transmitter.[68,69] Furthermore, certain receptor cells appear to take up these amino acids selectively.[70] No other substances so far tested have these specific effects; thus it has been proposed that one of these amino acids may serve as a receptor transmitter. However, objections to this view have been raised, and other potential photoreceptor transmitter candidates are being examined.[71] The inhibitory neurotransmitter, gamma-amino-butyric acid (GABA) has been observed by histochemical means to be present in the outer plexiform layer and taken up selectively by certain horizontal cells in some species,[72,73] electrophysiological evidence tends to support these observations.[74] There appear to be horizontal cells that do not use GABA as their transmitter, but the transmitter they do use is unknown.[73] At present, the nature and identity of the bipolar cell transmitter(s) are not known for any species.

In the inner plexiform layer, five neurotransmitter candidates—acetylcholine, GABA, glycine, dopamine, and an indoleamine, perhaps serotonin—have been identified by histochemical and, in some cases, other methods.[75–80] All these transmitters have been associated with amacrine cells; it has been demonstrated convincingly that some amacrine cells are capable of accumulating one or another of these substances. In addition, recent studies have shown that a substantial number of neuropeptides are present in many retinas, including substance P, somatostatin, enkephalin, vasointestinal peptide (VIP), glucagon, melanophore-stimulating hormone (alpha-MSH), and perhaps thyrotropin-releasing hormone (TRH).[81–83] Almost all these peptides have been localized by immunohistochemical methods to amacrine cells; the evidence thus far indicates that they are found in different morphological types of amacrine cells. As yet, there is no evidence that these neuropeptides coexist with the other neurotransmitter candidates in amacrine cells, suggesting that there may be as many as 12 or even more pharmacologically distinct types of amacrine cells in vertebrate retinas. The question of whether the neuropeptides serve as neurotransmitters or play some other role in the retina is under active investigation.

This pharmacological research, although still far from complete, has led to the important realization that each major type of retinal neuron may be subdivided on the basis of the neurotransmitter substance it contains and that distinct pharmacological subclasses of cells exist. It seems likely that these subclasses make specific and different synaptic connections within the plexiform layers, and mediate different functions within the retina. Evidence in support of this view has come from electron microscopic studies of the dopaminergic and indoleamine-accumulating amacrine cells in the rabbit retina.[17,84] The processes of the dopamine-containing amacrine cells make synapses only with other amacrine cells processes, whereas the processes of the indoleamine-accumulating amacrine cells make their junctions almost exclusively with bipolar terminals.

Finally, an area of basic retinal research that has not shown as much progress as the preceding, but is of great potential importance is that of retinal development. Investigators have long recognized that cell differentiation in retinas roughly proceeds centrifugally, and the ganglion cells, inner plexiform layer, and amacrine cells appear mature by light microscopy before the outer plexiform layer or photoreceptor cells.[85,86] However, studies with the electron microscope have now shown that this is not the case for the synaptic connections. Ribbon synapses of photoreceptors in the outer plexiform layer appear before those of the inner plexiform layer, and the bipolar cell ribbon synapses appear to be the last to mature.[87–92] Several studies have studied the changes in morphological appearance and density of synapses, particularly in the inner plexiform layer, as a function of age.[93,94] In a few cases, correlations have been made between the development of neural circuits within the retina and the maturation of the response properties of retinal cells, especially the receptive field properties of ganglion cells.[95] Very recent studies have described the development of neurotransmitter systems within the retina or within different retinal cell types, as a function of age.[96,97] However, little information is available concerning the factors underlying neurogenesis, synaptogenesis, or the development of retinal neurotransmitter systems. Since a number of inherited retinal lesions appear during development, such information is sorely needed.

RESEARCH NEEDS AND OPPORTUNITIES

The study of the retina has been especially fruitful over the past decade, and with adequate support,

rapid progress should continue in the future. The results of recent research point the way to additional studies that should keep retinal investigations at the forefront of vision and brain research.

Functional Organization of the Retina

Further analysis is needed of the "wiring" patterns within the plexiform layers of the retina to define precisely the various pathways for information flow through the retina. Sophisticated techniques such as computer reconstruction of serial electron micrographs can provide a level of detail thought unobtainable just a few years ago. Combined techniques such as serial section electron microscopy and the staining of individual neurons by electron-dense markers for neurotransmitter substances should provide additional information concerning the chemical-specific pathways that may be present within the retina. Studies of the differences between regions of the retina should be encouraged. Investigators still know very little about the macular and foveal regions of the primate eye, yet for human vision these retinal regions are of paramount importance.

Recent techniques such as freeze-fracture electron microscopy can provide further information about the molecular structure of the retinal synapses. As yet, only a few studies employing these methods have been conducted.[81] Virtually nothing is known about the factors underlying the development of retinal synapses; this is another area requiring substantial research.

Additional studies are needed to characterize and identify the physiological properties of single retinal neurons, especially in the mammalian retina. Intracellular staining for subsequent light-microscopic identification is routine today, but adapting this technique for electron microscopic examination is technically difficult. Much additional research on this problem is needed, for electron microscopy is essential in attempting to deduce how the responses of various types of neurons are produced by the synaptic inputs to those cells.

Other approaches and techniques appear to offer considerable promise for elucidating the properties of single retinal neurons, and should be encouraged. For example, it is possible to digest the retina apart with enzymes and isolate intact single neurons. This approach has been used in a few biochemical studies, but as yet little has been done to explore the physiological and pharmacological properties of isolated retinal neurons.[98] Such studies should produce important information concerning the membrane properties of the neurons, the receptors for neurotransmitter substances, and the ions involved in the maintenance and generation of potentials by the various retinal cells. The maintenance of isolated retinal cells in tissue culture is just beginning to be explored; it holds great promise for learning how retinal cells recognize and make synaptic junctions with one another.

Retinal Neurotransmission

Although a large number of substances have been proposed as retinal neurotransmitters, the evidence for them in many instances is not strong, and much more research is needed to establish the identity of the true retinal transmitters. Recent studies have suggested also that there are considerable species differences in the neurotransmitter used by one cell or another in various retinas, and it is possible that some retinal cells may be using neurotransmitter substances that have not yet been detected.

In addition to identifying the substance used at a particular retinal synapse, it is essential to explore the effects of the neurotransmitter substances on the postsynaptic cells. Such studies should include both the physiological and biochemical effects. For example, recent studies have shown that neurotransmitter substances in the brain not only interact with receptor sites on postsynaptic membranes to alter the permeability of the membrane to ions, but they may interact also with receptors that activate enzyme systems. The best characterized of the transmitter-activated enzymes is adenylate cyclase. This enzyme exists in the retina and may be activated by at least one of the known retinal transmitters, dopamine.[99,100] However, the role that this system plays in the retina or elsewhere in the brain is unknown.

A number of biochemical techniques are available to characterize further the properties of synapses in neural tissue. For example, it is possible to measure the binding of transmitter and related substances to receptor sites on the postsynaptic membranes of neurons and explore the molecular properties of the various binding sites. Such studies provided some of the earliest evidence for the presence of opiate-like transmitter substances (the enkephalins) within the brain. As noted above, the enkephalins are found in certain amacrine cells in the retinas of some species, but as yet what role such substances may play in the visual process is unknown.

In addition, receptor binding studies have shown that there are multiple types of receptors for single neurotransmitter substances. Little is known about the significance of this finding, but investigators have shown that one of the two dopamine receptors in certain regions of the brain is linked to adenylate cyclase, whereas the other is not. As yet, few studies of receptor binding have been conducted on the retina, although knowledge of its synaptic organization makes the retina an ideal tissue for such investigation.

Retinal Field Potentials and Visual Adaptation

An important area of retinal research not previously mentioned in this chapter is that of the nature of the retinal field potentials and visual adaptation. The best known of the retinal field potentials is the electroretinogram (ERG), a complex series of potentials that can be recorded from the intact eye.[101] The ERG is of considerable clinical importance, and research over the past two decades has shown that it arises partly from receptors, partly from the Müller (glial) cells of the retina, and partly from the pigment epithelium.[101–104] The underlying mechanisms for these potentials appear to involve shifts of small ions, K^+, Cl^-, and Na^+, across the membranes of the involved cells. Except for the photoreceptor currents, which give rise to the a-wave of the ERG, the details of how the other currents arise, resulting in the recorded potentials, are still not fully understood. Electrodes sensitive to various ions are now available and are being used to analyze the changes in ion concentrations which take place in the retina upon illumination. Because the ERG has important clinical relevance, such studies should be strongly encouraged (see Chapter 11, "Glial Cells and the Retinal Microenvironment").

Retinal adaptation may be a related problem. It is well known that the retina has the remarkable ability to alter its sensitivity depending on the prevailing illumination.[105] Everyone experiences this phenomenon upon entering a dimly-lit movie theater and finding that it takes several minutes for the eyes to gain enough sensitivity to "see" again. The mechanisms underlying visual adaptation reside almost exclusively within the retina. Research over the past decade has shown that much of visual adaptation occurs in the photoreceptors[106,107] but that retinal network mechanisms are also involved.[108] The nature of these mechanisms is at present completely unknown, but it has been proposed that alterations in the levels of K^+ at specific sites in the retina could be responsible for desensitizing certain of the retinal neurons in light.[109]

A number of night-blinding conditions are known, and understanding the mechanisms of visual adaptation in the normal eye may provide clues to the underlying defect in such diseases. For example, one such condition, Oguchi's disease, is characterized by an extremely slow recovery of light sensitivity following exposure of the eye to light. The receptors in patients with this disease show a normal pattern of light and dark adaptation, suggesting that the abnormality almost certainly resides in the retinal network and involves that adaptive mechanism.[110–111]

RECOMMENDATIONS

Based on the foregoing assessment of recent accomplishments, current activities, and research needs and opportunities in "Retinal Organization, Neurotransmission, and Adaptation," the Panel has made the following recommendations concerning research in this subprogram over the next five years. These have been grouped under two headings: Program Base and Program Development Priorities.

The Program Base includes areas of ongoing research where the current level of activity is considered adequate, or areas of ongoing research in which there may be great need for additional activity, but where, in the Panel's judgment, little or no opportunity (new methods or insights) exists at present to justify a significant expansion of effort. Nonetheless, additional applications for research grants in these areas may be funded if they are innovative and of very high quality as determined by the NIH peer review system.

Program Development Priorities include areas of ongoing research in which new knowledge and techniques offer particular opportunities for scientific progress, or promising new areas of research in which there is little or no support at present but where there is both great need and high potential for success. Such areas are judged to warrant significantly increased support over the next five years, provided that high quality applications for research grants in these areas are forthcoming.

Program Base

- Further analyze the pathways for information flow in the retina utilizing the full array of modern research techniques. Utilize immunohistochemical, monoclonal antibody, and other techniques to identify specific neurotransmitters and enzymes within neurons, and to identify specific cell types. More information about the macular and foveal regions of the human and subhuman primate eye is particularly needed.
- Characterize the physiological and pharmacological responses of retinal neurons. Emphasis should be placed on intracellular studies, especially in the mammalian retina, and on developing intracellular staining techniques which enable electron microscopic examination.
- Determine the physiological and metabolic basis of retinal network contributions to retinal field potentials and visual adaptation. Utilize this and other information gained from basic research to develop noninvasive (physiological and psychophysical) methods for studying retinal function (see Chapter 8, "Retinal Pigment Epithelium;"

Chapter 9, "Photoreceptors, Visual Pigments, and Phototransduction;" Chapter 11; and Chapter 13, "Noninvasive Techniques in the Study of Retinal Disorders").

Program Development Priorities

- Characterize retinal neurotransmitter substances, the associated metabolic pathways, and the physiological and biochemical effects of neurotransmitters on postsynaptic cells. Determine the types and molecular properties of neurotransmitter receptor sites using biochemical and pharmacological techniques.
- Elucidate the physiological, biochemical, and pharmacological properties of retinal neurons using isolated single cells and tissue culture techniques.
- Determine the factors underlying neurogenesis, synaptogenesis, the development of neurotransmitter systems, and the onset of retinal function.
- Investigate retinal metabolism, especially energy metabolism, and other biochemical processes critical for retinal function.

RESOURCE REQUIREMENTS

After reviewing current research grant support in each of these categories and assessing the need and potential for future development, the Panel has estimated the number of projects it believes are needed to carry out its recommendations in FY 1983. These estimates are shown in the table on the following page. For a discussion of the general basis and significance of these projections, see the "Summary" at the beginning of this report.

RESOURCE TABLE

RETINAL ORGANIZATION, NEUROTRANSMISSION, AND ADAPTATION

	No. of Grants FY 1981	Panel Recommendation FY 83	
		Add. Grants	Total Grants
Program Base			
A. Further analyze the pathways for information flow in the retina; identify specific neurotransmitters and enzymes within neurons and specific cell types.	13	1	14
B. Characterize the physiological and pharmacological responses of retinal neurons emphasizing intracellular studies.	18	0	18
C. Determine the basis of retinal network contributions to retinal field potentials and visual adaptation; develop noninvasive physiological and psychophysical tests of retinal function.	15	0	15
Program Development Priorities			
A. Characterize retinal neurotransmitters, receptors, the associated metabolic pathways, and the physiological and biochemical effects of neurotransmitters on postsynaptic cells.	9	2	11
B. Elucidate the physiological, biochemical, and pharmacological properties of retinal neurons using isolated single cells and tissue culture techniques.	3	2	5
C. Determine the factors underlying neurogenesis, synaptogenesis, the development of the neurotransmitter systems, and the onset of retinal function.	4	1	5
D. Investigate retinal metabolism, especially energy metabolism, and other biochemical processes critical for retinal function.	2	1	3
Subtotal Grants	**64**	**7**	**71**
(% of Program)	**(16)**	**(6)**	**(14)**
Total Estimated Cost	**$4,729,000**	**$2,726,000**	**$7,455,000**

REFERENCES

1. Cajal RY: *The Structure of the Retina.* SA Thorpe, M Glickstein (trans-ed), Springfield, IL, Charles C Thomas, 1972.
2. Hartline HK: The response of single optic nerve fibers of the vertebrate eye to illumination of the retina. *Am J Physiol* 121:400–415, 1938.
3. Barlow HB: Summation and inhibition in the frog's retina. *J Physiol (Lond)* 119:69–88, 1953.
4. Kuffler SW: Discharge patterns and functional organization of mammalian retina. *J Neurophysiol* 16:37–68, 1953.
5. Enroth-Cugell C, Robson JG: The contrast sensitivity of retinal ganglion cells of the cat. *J Physiol (Lond)* 187:517–552, 1966.
6. Maturana HR, Lettvin JY, McCulloch WS, et al: Anatomy and physiology of vision in frog (*Rana pipiens*). *J Gen Physiol* 43:129–175, 1960.
7. Barlow HB, Hill RM, Levick WR: Retinal ganglion cells responding selectively to direction and speed of image motion in the rabbit. *J Physiol (Lond)* 173:377–407, 1964.
8. Michael CR: Receptive fields of directionally selective units in the optic nerve of the ground squirrel. *Science* 152:1092–1094, 1965.
9. Michael CR: Receptive fields of single optic nerve fibers in a mammal with an all-cone retina. *J Neurophysiol* 31:249–282, 1968.
10. Kidd M: Electron microscopy of the inner plexiform layer of the retina in the cat and the pigeon. *J Anat* 96:179–188, 1962.
11. Missotten L: *The Ultrastructure of the Retina.* Brussels, Arscia Uitgaven NV, 1965.
12. Dowling JE, Boycott BB: Organization of the primate retina: Electron microscopy. *Proc R Soc Lond (Biol)* 166:80–111, 1966.
13. Kolb H: Organization of the outer plexiform layer of the primate retina: Electron microscopy of Golgi-impregnated cells. *Philos Trans R Soc Lond (Biol)* 258:261–283, 1970.
14. Stell WK: The morphological organization of the vertebrate retina, in Fuortes MGF (ed): *Handbook of Sensory Physiology*, vol 7, pt 2. Berlin, Springer-Verlag, 1972, pp 111–213.
15. Kolb H, Famiglietti EV: Rod and cone pathways in the inner plexiform layer of cat retina. *Science* 186:47–49, 1974.
16. Dowling JE, Ehinger B: Synaptic organization of the interplexiform cells of the goldfish retina. *Science* 188:270–273, 1975.
17. Ehinger B, Holmgren I: Electron microscopy of the indoleamine-accumulating neurons in the retina of the rabbit. *Cell Tissue Res* 197:175–194, 1979.
18. Dowling JE: Synaptic organization of the frog retina: An electron microscopic analysis comparing the retinas of frogs and primates. *Proc R Soc Lond (Biol)* 170:205–227, 1968.
19. Sjöstrand FS: Ultrastructure of retinal rod synapses of the guinea pig eye as revealed by three-dimensional reconstructions from serial sections. *J Ultrastruct Res* 2:122-170, 1958.
20. Stell WK: Correlation of retinal cytoarchitecture and ultrastructure in Golgi preparations. *Anat Rec* 153:389–397, 1965.
21. Lasansky A: Basal junctions at synaptic ending of turtle visual cells. *J Cell Biol* 40:577–581, 1969.
22. Dowling JE, Brown JE, Major D: Synapses of horizontal cells in rabbit and cat retinas. *Science* 153:1639–1641, 1966.
23. Dowling JE, Werblin FS: Organization of retina of the mudpuppy, *Necturus maculosus*: I. Synaptic structure. *J Neurophysiol* 32:315–338, 1969.
24. Kolb H, West R: Synaptic connections of the interplexiform cell in the retina of the cat. *J Neurocytol* 6:155–179, 1977.
25. Kolb H: The inner plexiform layer in the retina of the cat: Electron microscopic observations. *J Neurocytol* 8:295–329, 1979.
26. Raviola E, Gilula NB: Intramembrane organization of specialized contacts in the outer plexiform layer of the retina. *J Cell Biol* 65:192–222, 1975.
27. Naka K-I: The horizontal cell. *Vision Res* 12:573–588, 1972.
28. Miller RF: The neuronal basis of ganglion cell receptive field organization and the physiology of amacrine cells, in Schmitt FO, Worden FG (eds): *The Neurosciences: Fourth Study Program.* Cambridge, MIT Press, 1979, pp 227–245.
29. Dubin M: The inner plexiform layer of the vertebrate retina: A quantitative and comparative electron microscopic analysis. *J Comp Neurol* 140:479–506, 1970.
30. West RW, Dowling JE: Synapses onto different morphological types of retinal ganglion cells. *Science* 178:510–512, 1972.
31. Famiglietti EV Jr, Kolb H: Structural basis for on and off center responses in retinal ganglion cells. *Science* 194:193–195, 1976.
32. Famiglietti EV Jr, Kanebo A, Tachibana M: Neuronal architecture of on and off pathways to ganglion cells in carp retina. *Science* 198:1267–1269, 1977.
33. Bortoff A: Localization of slow potential responses in the Necturus retina. *Vision Res* 4:626–627, 1964.
34. Werblin FS, Dowling JE: Organization of the retina of the mudpuppy, *Necturus maculosus*: II. Intracellular recording. *J Neurophysiol* 32:339–355, 1969.
35. Kaneko A: Physiological and morphological identification of horizontal, bipolar, and amacrine cells in the goldfish retina. *J Physiol (Lond)* 207:623–633, 1970.
36. Baylor DA, Fuortes MGF, O'Bryan PM: Receptive fields of single cones in the retina of the turtle. *J Physiol (Lond)* 214:265–294, 1971.
37. Matsumoto N, Naka K-I: Identification of intracellular responses in the frog retina. *Brain Res* 42:59–71, 1972.
38. Naka K-I: Neuronal circuitry in the catfish retina. *Invest Ophthalmol Vis Sci* 15:926–934, 1976.
39. Nelson R, Kolb H, Famiglietti EV Jr, et al: Neural responses in the rod and cone systems of the cat retina: Intracellular records and Procion stains. *Invest Ophthalmol Vis Sci* 15:946–953, 1976.
40. Nelson R, Famiglietti EV Jr, Kolb H: Intracellular staining reveals different levels of stratification for

on- and off-center ganglion cells in cat retina. *J Neurophysiol* 41:472–483, 1978.
41. Svaetichin G, MacNichol EF: Retinal mechanisms for chromatic and achromatic vision. *Ann NY Acad Sci* 74:385–404, 1958.
42. Tomita T: Electrophysiological study of the mechanisms subserving color coding in the fish retina. *Cold Spring Harbor Symp Quant Biol* 30:559–566, 1965.
43. Baylor DA, Fuortes MGF: Electrical responses of single cones in the retina of the turtle. *J Physiol (Lond)* 207:77–92, 1970.
44. Schwartz EA: Responses of bipolar cells in the retina of the turtle. *J Physiol (Lond)* 236:211–224, 1974.
45. Trifonov YA, Byzov AL: The response of the cells generating S-potential on the current passed through the eye cup of the turtle. *Biofizika* 10:673–680, 1965.
46. Trifonov YA: Study of synaptic transmission between the photoreceptor and horizontal cell using electrical stimulation of the retina. *Biofizika* 13:809–817, 1968.
47. Dowling JE, Ripps J: Neurotransmission in the distal retina: The effect of magnesium on horizontal cell activity. *Nature* 242:101–103, 1973.
48. Ripps H, Shakib M, MacDonald ED: Peroxidase uptake by photoreceptor terminals of the skate retina. *J Cell Biol* 70:86–96, 1976.
49. Naka K, Witkovsky P: Dogfish ganglion cell discharge resulting from extrinsic polarization of the horizontal cell. *J Physiol (Lond)* 223: 449–460, 1972.
50. Miller RF, Dacheux R: Synaptic organization and ionic basis of on and off channels in mudpuppy retina. *J Gen Physiol* 67:639–690, 1976.
51. Toyoda J, Hashimoto H, Ohtsu K: Bipolar-amacrine transmission in the carp retina. *Vision Res* 13:295–307, 1973.
52. Werblin FS: Response of retinal cells to moving spots: Intracellular recording in *Necturus maculosus*. *J Neurophysiol* 33:342–351, 1970.
53. Caldwell JH, Daw NW, Wyatt HJ: Effects of picrotoxin and strychnine on rabbit retinal ganglion cells: Lateral interactions for cells with more complex receptive fields. *J Physiol (Lond)* 276:277–298, 1978.
54. Crawford AC, McBurney RN: On the elementary conductance event provided by L-glutamate and quanta of the natural transmitter at the neuromuscular junctions of *Maia squinado*. *J Physiol (Lond)* 258:205–225, 1976.
55. Katz B, Miledi R: The statistical nature of the acetylcholine potential and its molecular components. *J Physiol (Lond)* 224:665–699, 1972.
56. Schwartz EA: Comparison of the voltage noise and the response to one photon in the rods of the turtle retina, in Barlow HB, Fatt P (eds): *Vertebrate Photoreception*. New York, Academic Press, 1977, pp 323–336.
57. Ashmore JF, Falk G: Absolute threshold of rod bipolar cells in a dark-adapted retina. *Nature* 263:248–249, 1976.
58. Barlow HB: Retinal and central factors in human vision limited by noise, in Barlow HB, Fatt P (eds): *Vertebrate Photoreception*. New York, Academic Press, 1977, pp 337–358.
59. Toyoda JI: Membrane resistance changes underlying the bipolar cell response in the carp retina. *Vision Res* 13:283–294, 1973.
60. Werblin FS: Anomalous rectification in horizontal cells. *J Physiol (Lond)* 244:639–657, 1975.
61. Kaneko A, Shimazaki H: Effects of external ions on the synaptic transmission from photoreceptors to horizontal cells in the carp retina. *J Physiol (Lond)* 252:509–522, 1975.
62. Piccolino M, Gerschenfeld H: Characteristics and ionic processes involved in feedback spikes of turtle cones. *Proc R Soc Lond (Biol)* 206:439–463, 1980.
63. Heuser JE, Reese TS, Dennis MJ, et al: Synaptic vesicle exocytosis captured by quick freezing and correlated with quantal transmitter release. *J Cell Biol* 81:275–300, 1979.
64. Heuser JE, Salpeter SR: Organization of acetylcholine receptors in quick-frozen, deep etched and rotary replicated Torpedo synaptic membrane. *J Cell Biol* 82:150–173, 1979.
65. Dewey MM, Blaisie JK, Davis PK, et al: Localization of rhodopsin antibody in the retina of the frog. *J Mol Biol* 39:395–405, 1969.
66. McLaughlin BJ, Wood JG: The localization of concanavalin A binding sites during photoreceptor synaptogenesis in the chick retina. *Brain Res* 119:57–71, 1977.
67. Pfenninger K, Akert K, Moor H, et al: The fine structure of freeze-fractured presynaptic membranes. *J Neurocytol* 1:129–149, 1972.
68. Cervetto L, MacNichol EF: Inactivation of horizontal cells in turtle retina by glutamate and asparate. *Science* 178:767–768, 1972.
69. Wu SM, Dowling JE: L-aspartate: Evidence for a role in cone photoreceptor synaptic transmission in the carp retina. *Proc Natl Acad Sci USA* 75:5205–5209, 1978.
70. Lam DMK, Hollyfield JG: Localization of putative amino acid transmitters in the human retina. *Exp Eye Res* 31:792–832, 1980.
71. Gerschenfeld HM, Piccolino M: Pharmacology of the connections of cones and L-horizontal cells in the vertebrate retina, in Schmitt FO, Worden FG (eds): *The Neurosciences: Fourth Study Program*. Cambridge, MA, MIT Press, 1979, pp 213–226.
72. Lam DMK, Steinman L: The uptake of gamma-aminobutyric acid in the goldfish retina. *Proc Natl Acad Sci USA* 68:2777–2781, 1971.
73. Marc RE, Stell WK, Bok D, et al: GABA-ergic pathways in the goldfish retina. *J Comp Neurol* 182:221–246, 1978.
74. Wu SM, Dowling JE: Effects of GABA and glycine on the distal cells of the cyprinid retina. *Brain Res* 199:401–414, 1980.
75. Graham LT Jr: Comparative aspects of neurotransmitters in the retina, Davson H, Graham LT Jr (eds): *The Eye*, vol 6. New York, Academic Press, 1974, pp 283–342.
76. Neal MJ: Acetylcholine as a retinal transmitter substance, in Bonting SL (ed): *Transmitters in the Visual Process*. Oxford, Pergamon Press, 1976, pp 127–143.

77. Ehinger B: Biogenic monoamines as transmitters in the retina, in Bonting SL (ed): *Transmitters in the Visual Process.* Oxford, Pergamon Press, 1976, pp 145–163.
78. Voaden MJ: Gamma-aminobutyric acid and glycine as retinal neurotransmitters, in Bonting SL (ed): *Transmitters in the Visual Process.* Oxford, Pergamon Press, 1976, pp 107–125.
79. Masland RH, Livingstone CJ: Effect of stimulation with light on synthesis and release of acetylcholine by an isolated mammalian retina. *J Neurophysiol* 39:1210–1219, 1976.
80. Masland RH, Mills JW: Autoradiographic identification of acetylcholine in the rabbit retina. *J Cell Biol* 83:159–178, 1979.
81. Brecha N, Karten HJ, Laverack C: Enkephalin-containing amacrine cells in the avian retina: Immunohistochemical localization. *Proc Natl Acad Sci USA* 76:3010–3014, 1979.
82. Karten HJ, Brecha N: Localization of substance P immunoreactivity in amacrine cells of the retina. *Nature* 283:87–88, 1980.
83. Yamada T, Marshak D, Basinger S, et al: Somatostatin-like immunoreactivity in the retina. *Proc Natl Acad Sci USA* 77:1691–1695, 1980.
84. Dowling JE, Ehinger B: Synaptic organization of the dopaminergic neurons in the rabbit retina. *J Comp Neurol* 180:203–220, 1978.
85. Noell WK: Differentiation, metabolic organization, and viability of the visual cell. *Arch Ophthalmol* 60:702–733, 1958.
86. Cajal RY: *Studies on Vertebrate Neurogenesis.* Guth L (trans-ed), Springfield, Charles C Thomas, 1970.
87. Olney JW: An electron microscopic study of synapse formation, receptor outer segment development, and other aspects of developing mouse retina. *Invest Ophthalmol Vis Sci* 7:250–268, 1968.
88. Nilsson SEG: Receptor cell outer segment development and ultrastructure of the disk membranes in the retina of the tadpole (*Rana pipiens*). *J Ultrastruct Res* 11:581–620, 1964.
89. Weidman TA, Kuwabara T: Postnatal development of the rat retina: An electron microscopic study. *Arch Ophthalmol* 79:470–484, 1968.
90. Smelser GK, Ozanics V, Rayborn M, et al: Retinal synaptogenesis in the primate. *Invest Ophthalmol Vis Sci* 13:340–361, 1974.
91. Blanks JC, Adhinolfi AM, Lolley RN: Synaptogenesis in the photoreceptor terminal of the mouse retina. *J Comp Neurol* 156:81–93, 1974.
92. Cragg BG: The development of synapses in the visual system of the cat. *J Comp Neurol* 160:147–166, 1975.
93. Fisher LJ: Changes during maturation and metamorphosis in the synaptic organization of the tadpole inner plexiform layer. *Nature* 235:391–393, 1972.
94. McArdle CB, Dowling JE, Masland RH: Development of outer segments and synapses in the rabbit retina. *J Comp Neurol* 175:253–274, 1977.
95. Masland RH: Maturation of function in the developing rabbit retina. *J Comp Neurol* 175:275–286, 1977.
96. Lam DMK, Fung SC, Kong YC: Postnatal development of GABA-ergic neurons in the rabbit retina. *J Comp Neurol* 193:89–102, 1980.
97. Kong YC, Fung SC, Lam DMK: Postnatal development of glycinergic neurons in the rabbit retina. *J Comp Neurol* 193:1127–1135, 1980.
98. Lam DMK: Synaptic chemistry of identified cells in the vertebrate retina. *Cold Spring Harbor Symp Quont Biol* 40:571–579, 1975.
99. Brown JH, Makman MH: Stimulation by dopamine of adenylate cyclase in retinal homogenates and of adenosine 31,51-cyclic monophosphate formation in intact retina. *Proc Natl Acad Sci USA* 69:539–543, 1972.
100. Watling KJ, Dowling JE: Dopaminergic mechanisms in teleost retina. *J Neurochem* 36:559–579, 1981.
101. Brown KT: The electroretinogram: Its components and their origin. *Vision Res* 8:633–677, 1969.
102. Miller RF, Dowling JE: Intracellular responses of the Müller (glial) cells of the mudpuppy retina: Their relation to b-wave of the electroretinogram. *J Neurophysiol* 33:323–341, 1970.
103. Noell WK: The origin of the electroretinogram. *Am J Ophthalmol* 28:78–90, 1954.
104. Oakley B, Green DG: Correlation of light-induced changes in retinal extracellular potassium concentration with the c-wave of the electroretinogram. *J Neurophysiol* 39:1117–1133, 1976.
105. Rushton WAH: Visual adaptation. *Proc R Soc Lond (Biol)* 162:20–46, 1965.
106. Boynton RM, Whitten DN: Visual adaptation in monkey cones: Recordings of late receptor potentials. *Science* 170:1423–1426, 1970.
107. Kleinschmidt J, Dowling JE: Intracellular recordings from gecko photoreceptors during light and dark adaptation. *J Gen Physiol* 66:617–648, 1975.
108. Green DG, Dowling JE, Siegal IM, et al: Retinal mechanisms of visual adaptation in the skate. *J Gen Physiol* 65:483–502, 1975.
109. Dowling JE, Ripps H: Potassium and retinal sensitivity. *Brain Res* 107:617–622, 1976.
110. Carr RE, Ripps H: Rhodopsin kinetics and rod adaptation in Oguchi's disease. *Invest Ophthalmol Vis Sci* 6:426–436, 1967.
111. Dowling JE, Ripps H: From sea to sight. *Oceanus* 19:28–33, 1976.

11

GLIAL CELLS AND THE RETINAL MICROENVIRONMENT

INTRODUCTION

IN THE RETINA, as in other parts of the nervous system, the plasma membrane that invests each neuron houses molecular machinery vital to the cell's development and function. Specialized sites along this structure control the inward and outward mobility of ions important in electrogenesis, bind hormones and transmitter agents that modulate cellular behavior, and contain the energy-dependent pumps that subserve transmembrane transport. Although the neuronal membrane continues to be of special interest, there is increasing awareness that the functional integrity of membrane processes relies heavily on the physicochemical properties of the surrounding environment. [1]

Nerve cells are exquisitely sensitive to extracellular concentrations of electrolytes and metabolites; minute changes in their environment can alter the threshold of excitability in adjacent neurons and thereby influence the sensory message. Although unique homeostatic mechanisms buffer the neuronal elements against extreme fluctuations, controlled alterations of the external media may represent signals by which the activities of groups of nerve cells are integrated. [2] Thus, the microenvironment of the retina can be considered a dynamic milieu whose changing pattern of inhomogeneities modifies and regulates neuronal behavior. [3]

Although difficult to define precisely, the retinal microenvironment consists mainly of the cellular elements and fluid compartments within which the retinal neurons reside. In the most distal parts of the retina, the subretinal space and pigment epithelial processes that ensheath the receptor outer segments are the major constituents of the extraneural retina. Throughout the remainder of its depth, extending from the receptor inner segments to the vitreal border of the retina, an elaborate framework of glial cells provides a highly ordered satellite system for the retinal neurons. [4]

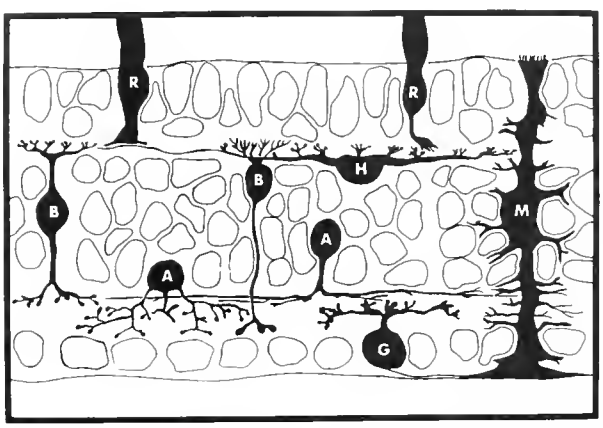


FIGURE. Schematic illustration of the principal cell types found in the vertebrate retina. The drawing is based on Golgi-impregnation of cells in the mudpuppy retina. The Müller cell (M) descends from the external limiting membrane at the level of the photoreceptors (R) to the internal limiting membrane. In its course it extends cytoplasmic sheets that surround the processes and cell bodies of the bipolar (B), amacrine (A), ganglion (G), and horizontal (H) cells. (Courtesy of JE Dowling.)

The most prominent glial element is the Müller cell (Figure), which traverses the layers of the retina in a radial direction from the internal to the external limiting membrane, projecting beyond the

latter to the outer nuclear layer where it envelops the perikariya of the rods and cones in honeycomb-like fibrillar "baskets."[5] Fine lamellar processes extend laterally from the Müller cells to enclose the neuronal elements of the inner nuclear layer and intercalate the synaptic terminals of the inner and outer plexiform layers. Other forms of neuroglia are found in the inner plexiform and nerve fiber layers of the retina (astrocytes), and in intimate relationship with the retinal vessels.

Far from being passive components of this architectural complex, the retinal glia serve important nutritive, ionic, and metabolic functions. In addition to providing structural support, glial cells have been implicated in the control of the potassium concentration of the extracellular fluid, degradation of some synaptically released neurotransmitter agents,[6-8] removal of metabolites,[9-11] and nourishment of the neuronal elements.[12]

Recent studies on the functional properties of Müller cells have indicated that they are well-suited to these roles. The Müller cell, like glial elements elsewhere in the nervous system, is highly permeable to potassium ions[13,14] and may actively transport K^+ released by neuronal activity into the extracellular space.[15] In addition, these cells contain high specific activities of the transmitter-degrading enzymes acetylcholinesterase and GABA-transaminase, and also two presumably glial-specific enzymes, carbonic anhydrase[10,16] and glutamine synthetase.[17] Moreover, the presence of numerous mitochondria in selected zones of the Müller cell cytoplasm suggests a nutritive function, perhaps by transferring high-energy compounds to the nerve cells.[12]

Interposed between the neuron and glia is the extracellular space, which contains an intercellular matrix that has various properties related to its polyanionic composition.[18] Difficulties in isolating and preserving the extracellular matrix have precluded quantitative analysis. However, its structure has been visualized by high voltage electron microscopy,[19] and some of the important molecular entities have been identified in brain tissue. Among the substances that have attracted the most attention are the glycosaminoglycans, glycoproteins, and gangliosides, many of which are associated with the nerve-cell coat but may be present also in the extracellular space.[20] Some of the possible functions of these macromolecules include: recognition and adhesion during neural development and plasticity, ionic buffering by absorption and release, and control of the mobility of bioactive molecules and ions (for example, the calcium-binding properties of sialic acid[21]).

Further insights into the functional significance of the retinal microenvironment have been obtained from studies of the electrophysiology of the retinal glia and the ionic shifts in the extracellular space resulting from neural activity. Of particular interest is the interaction of the microenvironment with local neuronal circuits. It has been suggested that changes in extracellular potassium (K^+) resulting from neural activity may in turn influence the sensitivity and adaptive behavior of retinal neurons[22] and may generate radial currents that give rise to the b-wave and other components of the electroretinogram.[23-25]

SUBPROGRAM OBJECTIVES

- To identify the mechanisms that control the dynamic extracellular milieu of the normal retina and disturb the normal milieu under pathological conditions.
- To determine the specialized membrane properties that enable the unidirectional channeling of metabolites and other substances through the cell and surrounding media (for example, the functional polarization that determines the nature and direction of retinal glia, including glial contributions to the electroretinogram, and the possibility that the electrical activity of these elements influences the information processing of neural circuits.
- To determine the role of the glial elements in neuronal metabolism, for example, in terminating the actions of neurotransmitters and transporting to nerve endings the precursors required to replenish the supply of transmitter substances.
- To determine the role of neuroglia in retinal development and the regenerative response of injured nerve cells.

OVERVIEW OF CURRENT RESEARCH SUPPORT

In FY 1981, 6 National Eye Institute grants at a total cost of $437,000 supported research on Müller cells and the retinal environment. In addition, a number of other NEI-supported research projects in retinal physiology were broad enough to encompass this area of interest. Most of the research cited above resulted from NEI-sponsored research into retinal metabolism and cellular physiology.

RECENT ACCOMPLISHMENTS

In recent years, important new concepts have been developed about the high degree of interdependence between nerve cells and their surroundings. Owing to the close packing of nerve cells in the nuclear and synaptic layers of the retina, the extracellular space and glial elements acquire the potential to act as modulators of the chemical substances that influence neuronal behavior,[23,24] as communication channels between neurons,[25] and as the source of transretinal potentials useful in the diagnostic evaluation of retinal diseases.[26–29] Evidence that such functions are attributable to non-neuronal elements has been culled largely from studies that have taken advantage of the many technical innovations in biochemical and biophysical methods; indeed, some of the problems recently addressed have become amenable to study only in the past decade. For example, the improved resolution provided by new histochemical methods has effectively demonstrated the interaction between neuron and glia in the uptake, inactivation, and reformation of amino acid transmitter candidates in the retina.[30,31] A number of studies have shown that glial cells, whether in the retina or in culture, contain high affinity uptake systems for GABA, L-glutamate, and L-aspartate.[32,33]

The fact that glutamine synthetase and the GABA-degrading enzyme GABA-transaminase have been localized within Müller cells by autoradiographic and immunohistochemical techniques tends to support the view that a GABA-glutamate-glutamine cycle, involving neurons, glia, and the extracellular space, may be present in the retina. In this view, the uptake by Müller cells of neuronally-released glutamate and GABA terminates the transmitter action of these agents. Within the Müller cell, they are degraded or converted to glutamine by the action of glutamine synthetase. Glutamine, which is freely diffusable, then leaves the Müller cell and becomes available to neurons for replenishing glutamate and GABA. It is not yet known whether the retinal nerve cells utilize amino acids as neurotransmitters, but if a system such as this operates in the retina, the Müller cell forms an indispensable element in maintaining the functional integrity of the retinal neurons.

Further evidence that the glia play a role in restoring extracellular conditions favorable to neuronal functioning stems from research with ion-specific microelectrodes. Earlier studies by Kuffler and associates[13] showed that glial cells are highly permeable to K^+ and can be considered to react to ionic changes like a potassium electrode. Accordingly, they suggested that the glial syncytium may serve as a passive conveyor (that is, spatial buffer) of K^+, removing excessive K^+ released by depolarizing neurons. The alternative view, that glial cells may clear potassium by active transport, is supported by evidence for a Na^+-K^+ activated ATPase, and oxygen-dependent and anaerobic energy-yielding enzyme systems in glial cells.[10] The availability of energy-yielding reactions that take place despite a shortage of oxygen may be required for the removal of K^+ in hypoxic emergencies arising from impaired blood flow. In either event, results obtained with ion-selective electrodes[34] have shown that the changes in extracellular K^+ that occur with neuronal activity or spreading depression result in a rise in the potassium concentration within Müller cells.[14,35] Moreover, there is growing evidence that light-evoked K^+ fluxes are responsible for the production of radial currents that contribute to the waveform of the electroretinogram (ERG).[28,29,36]

Although the ERG has long been one of the most valuable noninvasive techniques in the diagnosis of retinal diseases, its clinical usefulness has not yet been fully realized. Perhaps the primary obstacle is the uncertainty surrounding the origins of the various potentials that contribute to its complex waveform. Clearly, if this were known, the site and nature of defective function might be inferred from the selective loss of one or another of the component potentials. Indeed, some noteworthy advances have been made, and the information has been used to improve our understanding of clinical problems.[37,38]

The initial portion of the a-wave of the ERG is the extracellular expression of the light-induced conductance change across the photoreceptor membrane,[39] whereas the b-wave presumably is a glial-cell potential that results from the activity of cells in the inner nuclear layer.[29,40,41] The research on the origins of the c-wave leaves little doubt that this potential is generated across the apical membrane of the retinal pigment epithelium in response to a slow, light-induced fall in the extracellular potassium concentration surrounding the visual cells.[42,43]

Because the b-wave is the principal component of the ERG response and reflects accurately the sensitivity of the visual system under a variety of experimental conditions, it has been particularly useful in studies of retinal disorders; it is of value to the clinician in revealing the loss of rod vision in night-blinding diseases,[38,44] defective photopic mechanisms in cone dysfunction syndromes,[45] and the early onset of widespread degeneration in retinitis pigmentosa.[37] Nevertheless, the cellular basis of this potential is still uncertain, and should be clarified, in view of the limited number of objective, quantitative, noninvasive methods available for the study of human retinal diseases.

RESEARCH NEEDS AND OPPORTUNITIES

Powerful new tools are available for studying the structure and functional role of the retinal cell environment, and a quantitative description of the homeostatic mechanisms that regulate the ionic composition of the extracellular milieu is needed. Ion-specific microelectrodes afford an opportunity to measure the ionic fluxes associated with normal function and abnormal processes.[14,46,47] The conditions that result in such brain phenomena as seizures and spreading depression may exist in the diseased retina as a consequence of abnormal ionic shifts induced by photic exposure.[48,49] In addition, extracellular potassium gradients appear to be a principal factor in the production of slow potentials in the retina. A comprehensive understanding of how these potentials arise is not yet available, but their clinical potential has become increasingly evident.

The unique structure of glial cells, particularly in the regions of close apposition with neuronal elements, may have important implications for their functional significance.[50] High voltage electron microscopy[51] and freeze-fracture studies[50] can help to reveal regional heterogeneities along the length of the Müller cell, which may have relevance for understanding neuron-glia interactions, inactivation of neurotransmitters, and interpretation of studies of neuronal degeneration. Chemically induced neuronal degeneration (for example, with kainic acid) leads not only to the loss of neurons but also to a glial proliferation or gliosis.[52] The structure and biochemical properties of the proliferating astroglia may provide clues to the factors inhibiting neuronal regeneration (see Chapter 12, "Rescue and Regeneration of Neurons in the Optic Nerve and Retina").

Enzymatic tissue dissociation has proved useful for obtaining isolated populations of glial cells, which can be maintained for prolonged periods in tissue culture.[53] The cells retain their high capacity to accumulate actively specific amino acids[54] and provide a useful system in which to investigate the morphological and chemical changes produced by gliotoxic agents such as alpha-aminoadipate[55,56] and 6-aminonicotinamide,[56,57] energy metabolism of glial tissue,[58] and various drugs that may interact with and regulate Müller cell function.[59,60]

RECOMMENDATIONS

Based on the foregoing assessment of recent accomplishments, current activities, and research needs and opportunities in "Glial Cells and the Retinal Microenvironment," the Panel has made the following recommendations concerning research in this subprogram over the next five years. These have been grouped under two headings: Program Base and Program Development Priorities.

The Program Base includes areas of ongoing research where the current level of activity is considered adequate, or areas of ongoing research in which there may be great need for additional activity, but where, in the Panel's judgment, little or no opportunity (new methods or insights) exists at present to justify a significant expansion of effort. Nonetheless, additional applications for research grants in these areas may be funded if they are innovative and of very high quality as determined by the NIH peer review system.

Program Development Priorities include areas of ongoing research in which new knowledge and techniques offer particular opportunities for scientific progress, or promising new areas of research in which there is little or no support at present but where there is both great need and high potential for success. Such areas are judged to warrant significantly increased support over the next five years, provided that high quality applications for research grants in these areas are forthcoming.

Program Base

- Conduct studies to isolate and identify the contribution of the Müller cells to the various components of the transretinal ERG and to assess changes in glial cell properties associated with neuronal degeneration.

Program Development Priorities

- Develop and utilize ion-specific electrodes for quantitative analysis of the alterations in extracellular and intracellular activities of essential ions throughout the retina in light and dark and for studies on the active and passive mechanisms of ionic buffering.
- Expand the use of cell separation and culture techniques to isolate and maintain viable glial cell populations for metabolic and pharmacological studies and for biochemical studies of neurotransmitter uptake.

RESOURCE REQUIREMENTS

After reviewing current research grant support in each of these categories and assessing the need and potential for future development, the Panel has

estimated the number of projects it believes are needed to carry out its recommendations in FY 1983. These estimates are shown in the table on the following page. For a discussion of the general basis and significance of these projections, see the "Summary" at the beginning of this report.

RESOURCE TABLE

GLIAL CELLS AND THE RETINAL MICROENVIRONMENT

	No. of Grants FY 1981	Panel Recommendation FY 83	
		Add. Grants	Total Grants
Program Base			
A. Conduct studies to isolate and identify the contribution of the Müller cells to the various components of the transretinal ERG and assess changes in glial cell properties associated with neuronal degeneration.	3	1	4
Program Development Priorities	1	1	2
A. Develop and utilize ion-specific electrodes for quantitative analysis of extracellular and intracellular activities of essential ions throughout the retina and for studies of mechanisms of ionic buffering.			
B. Expand the use of cell-separation and culture techniques to isolate and maintain viable glial cell populations for metabolic and pharmacological studies and for biochemical studies of neurotransmitter uptake.	2	1	3
Subtotal Grants	**6**	**3**	**9**
(% of Program)	**(1)**	**(3)**	**(2)**
Total Estimated Cost	**$437,000**	**$508,000**	**$945,000**

REFERENCES

1. Hyden H, Pigon A: A cytophysiological study of the functional relationship between oligodendroglial cells and nerve cells of Deiter's nucleus. *J Neurochem* 6:57–72, 1960.
2. Somjen GG: Do potassium, glia and neurons interact?, in Woody CD, Brown KA, Crow TG, et al (eds): *Cellular Mechanisms Subserving Changes in Neuronal Activity*. UCLA Brain Information Service Research Report No 3, University of California, 1974.
3. Somjen GG: Electrophysiology of neuroglia. *Annu Rev Physiol* 37:163–190, 1975.
4. UGA S, Smelser GK: Comparative study of the fine structure of retinal Müller cells in various vertebrates. *Invest Ophthalmol Vis Sci* 12:434–448, 1973.
5. Sjöstrand FS: The ultrastructure of the inner segments of the retinal rods of the guinea pig eye as revealed by electron microscopy. *J. Cell Comp Physiol* 42:45-70, 1953.
6. Henn FA, Goldstein MN, Hamberger A: Uptake of the neurotransmitter candidate glutamate by glia. *Nature* 249:663–664, 1974.
7. Hyde JC, Robinson N: Localization of sites of GABA catabolism in the rat retina. *Nature* 248:432–433, 1974.
8. Balcar VJ, Borg J, Mandel P: High affinity uptake of L-glutamate and L-aspartate by glial cells. *J Neurochem* 28:87–93, 1977.
9. Kuwabara T, Cogan DG: Tetrazolium studies on the retina: I. Introduction and technique. *J Histochem Cytochem* 7:329–341, 1959.
10. Tower DB, Young OM: The activities of butyrylcholinesterase and carbonic anhydrase, the rate of anaerobic glycolysis, and the question of a constant density of glial cells in cerebral cortices of various mammalian species from mouse to whale. *J Neurochem* 20:269–278, 1973.
11. Ehinger B: Glial uptake of taurine in the rat retina. *Brain Res* 60:512–516, 1973.
12. Rasmussen KE: A morphometric study of the Müller cells, their nuclei and mitochondria, in the rat retina. *J Ultrastruct Res* 44:96–112, 1973.
13. Kuffler SW, Nicholls JG: The physiology of neuroglial cells. *Ergeb Physiol* 57:1–90, 1966.
14. Mori S, Miller WH, Tomita T: Müller cell function during spreading depression in frog retina. *Proc Natl Acad Sci USA* 73:1351–1354, 1976.
15. Heinemann U, Lux HD: Undershoots following stimulus-induced rises of extracellular potassium concentration in cerebral cortex of cat. *Brain Res* 93:63–76, 1975.
16. Musser GL, Rosen S: Localization of carbonic anhydrase activity in the vertebrate retina. *Exp Eye Res* 15:105–119, 1973.
17. Riepe RE, Norenberg MD: Müller cell localization of glutamine synthetase in rat retina. *Nature* 268:654–655, 1977.
18. Revel J-P, Ito S: The surface components of cells, in Davis BD, Warren L: *The Specificity of Cell Surfaces*. Englewood Cliffs, Prentice Hall, 1967, pp 211–234.
19. Rambourg A, Leblond CP: Electron microscopic observations on the carbohydrate-rich cell coat present at the surface of cells in the rat. *J Cell Biol* 32:27–53, 1967.
20. Abers RW, Kovel CJ: The interaction of gangliosides with cationic molecules. *Biochim Biophys Acta* 60:359–365, 1962.
21. Jacque LW, Brown EB, Barrett JM, et al: Sialic acid: A calcium-binding carbohydrate. *J Biol Chem* 252:4533–4539, 1977.
22. Dowling JE, Ripps H: Potassium and retinal sensitivity. *Brain Res* 107:617–622, 1976.
23. DeVellis J, Kukes G: Regulation of glial cell functions by hormones and ions: A review. *Tex Rep Biol Med* 31:271–293, 1973.
24. Hess HH, Embree LJ, Shein HM: Enzymatic control of sodium and potassium active transport in normal and neoplastic rodent astroglia. *Prog Exp Tumor Res* 17:308–317, 1972.
25. Nicholson C: Dynamics of the brain cell microenvironment. *Neurosci Res Program Bull* 18:177–322, 1980.
26. Oakley B II, Green DG: Correlation of light-induced changes in retinal extracellular potassium concentration with the c-wave of the electroretinogram. *J Neurophysiol* 39:1117–1133, 1976.
27. Witkovsky P, Dudek FE, Ripps H: Slow PIII component of the carp electroretinogram. *J Gen Physiol* 65:119–134, 1975.
28. Kline RP, Ripps H, Dowling JE: Generation of b-wave currents in the skate retina. *Proc Natl Acad Sci USA* 75:5727–5731, 1978.
29. Miller RF, Dowling JE: Intracellular responses of the Müller (glial) cells of mudpuppy retina: Their relation to b-wave of the electroretinogram. *J Neurophysiol* 33:323–341, 1970.
30. Neal MJ: Amino acid transmitter substances in the vertebrate retina. *Gen Pharmacol* 7:321–332, 1976.
31. Marshall J, Voaden MJ: Autoradiographic identification of the cells accumulating ^{3}H-gamma-aminobutyric acid in mammalian retinae: A species comparison. *Vision Res* 15:459–461, 1975.
32. Hansson HA: Müller's neuroglial cells in cultures of rabbit retina. *Exp Eye Res* 11:105–110, 1971.
33. Sarthy PV, Lam DMK: Biochemical studies of isolated glial (Müller) cells from the turtle retina. *J Cell Biol* 78:675–684, 1978.
34. Walker JL Jr: Ion specific liquid ion exchanger microelectrodes. *Anal Chem* 43:89–92, 1971.
35. Karwoski CJ, Proenza LM: Relation between Müller cell responses, a local transretinal potential, and potassium flux. *J Neurophysiol* 40:244–259, 1977.
36. Oakley B II: Potassium and the photoreceptor-dependent pigment epithelial hyperpolarization. *J Gen Physiol* 70:405–425, 1977.
37. Berson EL: Hereditary retinal diseases: Classification with the full-field electroretinogram. XIV ISCERG Symposium. *Doc Ophthalmol Proc Series* 13:149–171, 1977.
38. Ripps H: Night blindness and the retinal mechanisms of visual adaptation. *Ann R Coll Surg Engl* 58:222–232, 1976.

39. Penn RD, Hagins WA: Signal transmission along retinal rods and the origin of the electroretinographic a-wave. *Nature* 223:210–205, 1969.
40. Brown KT, Wiesel TN: Localization of origins of electroretinogram components by intraretinal recording in the intact eye. *J Physiol (Lond)* 158:257–280, 1961.
41. Faber D: *Analysis of Slow Transretinal Potentials in Response to Light*, thesis. State University of New York, Buffalo, 1969.
42. Steinberg RH, Schmidt R, Brown KT: Intracellular responses to light from cat pigment epithelium: Origin of the electroretinogram c-wave. *Nature* 227:728–730, 1970.
43. Oakley B II, Flaming DG, Brown KT: Effects of the rod receptor potential upon retinal extracellular potassium concentration. *J Gen Physiol* 74:713–737, 1979.
44. Carr RE, Ripps H, Siegel IM, et al: Rhodopsin and the electrical activity of the retina in congenital night blindness. *Invest Ophthalmol Vis Sci* 5:497–507, 1966.
45. Goodman G, Ripps H, Siegel IM: Cone dysfunction syndromes. *Arch Ophtholmol* 70:214–231, 1963.
46. Lux HD: Fast recording ion-specific microelectrodes: Their use in pharmacological studies in the CNS. *Neuropharmacology* 13:509–517, 1974.
47. Somjen GG, Rosenthal M, Cordingly G, et al: Potassium, neuroglia and oxidative metabolism in central gray matter. *Fed Proc* 35:1266–1271, 1976.
48. Miller RF, Dacheux R: Chloride sensitive mechanisms in the isolated retina-eyecup of the rabbit. *Brain Res* 90:329–334, 1975.
49. Ripps H, Mehaffey L III, Siegel IM: "Rapid regeneration" in the cat retina: A case for spreading depression. *J Gen Physiol* 77:335–346, 1981.
50. Rosenbluth J: Glial membrane specializations in extraparanodal regions. *J Neurocytol* 7:709–719, 1978.
51. Hama K, Mizukawa A, Kosaka T: Fine structure of the Müller cell revealed by high-voltage electron microscopy. *Sens Processes* 2:296–299, 1978.
52. Schousboe A, Drejer J, Divac I: Regional heterogeneity in astroglial cells: Implications of neuron-glia interactions. *Trends Neurosci* 3:13–14, 1980.
53. Sarthy PV, Lam DMK: Biochemical studies of isolated glial (Müller) cells from the turtle retina. *J Cell Biol* 78:675–684, 1978.
54. Balcar VJ, Borg J, Mandel P: High affinity uptake of L-glutamate and L-aspartate by glial cells. *J Neurochem* 28:87–93, 1977.
55. Olney JW, deGubareff T, Collins JF: Stereospecificity of the gliotoxic and anti-neurotoxic actions of alpha-aminoadipate. *Neurosci Lett* 19:277–282, 1980.
56. Szamier RB, Ripps H, Chappell RL: Changes in ERG b-wave and Müller cell structure induced by alpha-aminoadipic acid. *Neurosci Lett* 21:307–312, 1981.
57. Schaarschmidt W, Lierse W: Ultrastrukturelle Reaktion der multipotenten Glia in Kleinhirn der Ratter nach Behanklung mit 6-Aminonikotinamid. *Acta Anat* 93:184–193, 1975.
58. Tholey G, Roth-Schecter BJ, Mandel P: Development of glial cells in primary cultures: Energy metabolism and lactic dehydrogenase isoenzymes. *Neurochem Res* 5:847–854, 1980.
59. deVellis J, Kukes G: Regulation of glial cell functions by hormones and ions. *Tex Rep Biol Med* 31:271–293, 1973.
60. Henn FA, Henn SW: The psychopharmacology of astroglial cells. *Prog Neurobiol* 15:1–17, 1980.

12

RESCUE AND REGENERATION OF NEURONS IN THE OPTIC NERVE AND RETINA

INTRODUCTION

BLINDNESS CAN RESULT from injury to any part of the visual pathway, but the vast majority of cases result from conditions arising within the eye or in the optic nerve, which connects the eye to the brain. Destruction of the optic nerve makes an otherwise healthy eye useless, but complete loss of visual function can also result from far less drastic injuries. The traditional view has been that it is impossible to reconnect successfully a severed human optic nerve. Moreover, even if it were possible to repair a severed optic nerve physically, very complex structural and functional relationships would need to be reestablished for the patient to regain useful vision.

Surgeons have for many years been able to repair the human peripheral nervous system, but scar formation and other factors have proved to be severe obstacles to regeneration in the central nervous system.[1,2] In spite of its name, the optic nerve is not a peripheral nerve but, like the tracts of the spinal cord, a part of the central nervous system. Although the formidable biological problems of optic nerve and spinal cord regeneration are similar, the clinical applications of laboratory research results in these areas are likely to be quite different because (1) the spinal cord is a much more heterogeneous structure, (2) the functional criteria for successful regeneration are different, and (3) the prostheses that have been proposed to replace lost optic nerve functions are inherently more complex than those proposed to replace lost spinal and cord functions such as control of the urinary bladder or a limb. Thus, although research on optic nerve and spinal cord regeneration is likely to be mutually reinforcing, the problems in the two tissues are not identical.

A more immediate goal might be to apply the results of experimental studies of nerve growth and regeneration to the problem of preventing injured human optic nerves from undergoing degeneration. Such injuries commonly result from a single event such as trauma, infection, or temporary deficiencies of blood supply, or often from adverse extraocular conditions. More chronic injuries may result from glaucoma, exposure to toxins, or treatable intracranial tumors. There is usually a lag of about two months between the time of injury and the retrograde degeneration of individual optic nerve fibers and the retinal ganglion cells from which they arise. Although clinical studies have shown that functional improvement sometimes occurs spontaneously, this two-month period could provide an opportunity for treatment designed to rescue the optic nerve by redressing the balance between degeneration and regeneration within the unsevered but injured axons.

The terms "regeneration" and "rescue" require definition. Regeneration may be defined strictly as the complete restoration of morphological and functional integrity following severance of the optic nerve. A broader definition of regeneration would include instances in which growth of neural processes occurs to form new synaptic contacts with or without recovery of normal function. Rescue is used to indicate a therapeutic process promoting neuronal survival and functional recovery in an injured neuron.

Knowledge of rescue and regeneration in the central nervous system has progressed in parallel with the technical advances of modern biology. Phenomenologic observations of nervous tissue regeneration began about 1900, study of cellular mechanisms about 1950, and probing at the molecular level about 1970.[3] Additional research at all three levels will be required to generate hypotheses and experiments that could eventually lead to the development of treatments and clinical trials of their efficacy.

SUBPROGRAM OBJECTIVES

- To define the biology of retinal neurons, glial cells, and connective tissue cells in normal development and maintenance, and in response to injuries.
- To determine the physical and chemical factors that influence degeneration and that may promote rescue and regeneration.

OVERVIEW OF CURRENT RESEARCH SUPPORT

In FY 1981 the National Eye Institute supported 9 projects at a total cost of $602,000 in which research on the regeneration of the visual system was a primary concern. Seven of these projects, at a total cost of $466,000, were assigned to the Strabismus, Amblyopia, and Visual Processing program and two, at a total cost of $136,000, to the Retinal and Choroidal Diseases program. Substantial support for more general aspects of peripheral and central nervous system regeneration was provided by other components of the National Institutes of Health, particularly the National Institute of Neurological and Communicative Disorders and Stroke, and by the National Science Foundation.

Studies of the development and plasticity of the visual system, and of the nervous system generally, constitute a closely related research area of burgeoning opportunity and interest.

RECENT ACCOMPLISHMENTS

Phenomena of Regeneration and Recovery

It is encouraging that one model of successful central nervous system regeneration following axotomy has been found in the olfactory bulb of a nonhuman primate.[4] The factors that allow this region to be permeated by regrowing axons need to be determined. It is particularly significant that the regeneration occurred in mature monkeys rather than in prenatal animals in which regeneration and neogenesis can occur more readily. Clearly, this would be a useful model in which to investigate some of the variables (such as age and degree of injury) that influence the rate and extent of regeneration.

Important and related studies have been performed on lower vertebrates. In the goldfish, for example, the severed optic nerve will sprout neurites and eventually form functionally useful connections with the brain.[5,6] Research on goldfish may be particularly relevant because teleost fish have myelinated optic nerves; these are more similar to the optic nerves of higher vertebrates than are the unmyelinated axons of various amphibia which also can regenerate their optic nerves. Studies of other neural systems that regenerate successfully have led to partial identification of factors that contribute to or detract from the outcome, for example, conditioning lesions[7–10] and temperature.[11]

The role of the glial cells of the adult mammalian optic nerve in regeneration has been investigated by grafting a segment of the optic nerve into a severed peripheral nerve.[1] The optic nerve transplant did not support extensive reinnervation, although the peripheral nerve axons are capable of vigorous sprouting and would have regenerated successfully had the graft been a segment of peripheral nerve. Significantly, the optic nerve glia survived in this system and, although limited reinnervation occurred, were capable of stimulating peripheral nerve axon ensheathment and myelination. The glial cells of fish and amphibian optic nerve do not constitute such a barrier to optic nerve regrowth.

The role of the Schwann cells of peripheral axons has been studied by grafting the sciatic nerve into the thoracic spinal cord.[12] The sciatic nerve grafts receive regenerating intrinsic central nervous system axons that become myelinated. On occasion, regenerating axons were observed at the junction which had one heminode of Ranvier of central nervous system glial origin and one of peripheral nervous system Schwann cell origin.[13] When the regenerating axons re-encountered the CNS environment at the distal end of the graft, they ceased to

elongate. The timing of the grafts following spinal cord transection is an important variable.[14,15] Immediate grafting leads to the formation of a cyst-like cavity, whereas waiting one week prior to grafting produces a better result.

These studies suggest that an axonal signal for ensheathment and myelination exists that is common to both the central and peripheral nervous systems. The morphological evidence indicates that one of the important tasks for regeneration is passage of the neurite through the potentially obstructing sheath structures in the 1–2 mm segment adjacent to the cut end of the nerve or tract. The basal laminar structures of the peripheral nervous system Schwann cells allow the neurite to advance perhaps because the basal lamina can guide the advancing neuron while resisting the pressure exerted by the expanding terminal club. The central nervous system glial cells have no basal lamina and seem unable to perform this function, leading to continuous expansion and rupture of the terminal clubs.[14,16]

Collateral sprouting of local undamaged axons does occur in the central nervous system and can fill the postsynaptic sites on target cells that are vacated by degenerating axons.[17,18] The phenomenon of collateral sprouting indicates that the neuropil is at least capable of synapse formation after injury.[17–19] In the developing central nervous system, glial processes guide the migration of some axons, and in some experimental models, glial cells have been shown to have surface grooves that guide the growth of the developing axon.

Even in the peripheral nervous system, the pathway problem has not been entirely solved, and recent studies have suggested ways that nerve repair can be enhanced using synthetic materials.[20] Sutured nerves have a disorganized pattern of regeneration, which can be minimized by holding the cut ends together in a hypoantigenic synthetic collagen membrane tube.[20] Similar synthetic materials might facilitate orderly repair at the interface of severed CNS neurons and also be used to form part of a drug delivery system.

Axon-sheath cell interactions are important for conducting impulses through a neuron. Congenital deficiencies of myelination and defective remyelination have gained attention recently, and the demyelinating effects of multiple sclerosis are well known. A number of strains of myelin-deficient mice have been identified and studied,[2] and chimeras between dystrophic and normal mouse embryos have been developed. Axo-glial membrane specializations occurring in demyelinated spinal cord lesions in guinea pigs with experimental allergic encephalitis (EAE) and in a human with multiple sclerosis have been analyzed.[21] In the multiple sclerosis case the axons and glia exhibited desmosome-like specializations but lacked the synapse-like specializations and gap junctions seen in the spinal cord of the guinea pig EAE model.[21] However, in the guinea pig model the demyelinated lesions of the optic nerves showed no axo-glial membrane specializations. It has been speculated that the formation of these specializations requires the presence of gray matter which is present in the spinal cord but not in the optic nerve.[21]

Freeze fracture of tissues has contributed to understanding of axo-glial membrane interactions, and cytochemical techniques have revealed a filamentous subaxolemmal undercoating in the region of the node of Ranvier.[2] One of the most relevant problems in regeneration is the nature of the stimuli that both cause and curtail the proliferation of sheath cells. The initiation and time course of mitosis of non-neuronal cells has been followed by the incorporation of (^{3}H) thymidine.[22] Curtailment of growth is important to prevent the overgrowth of redundant sheath cell populations. Stimuli that have been preliminarily identified include the axons themselves,[2] extracts of pituitary gland and brain,[2] and cyclic AMP induction.[23]

Unfortunately, most of these studies involve Schwann cells of the peripheral nervous system rather than oligodendrocytes, which are more relevant to optic nerve regeneration. In adult mammalian central nervous system regeneration, most of the dividing cells are astrocytes and microglia, not oligodendrocytes. Because nerve impulse conduction depends on the location of the node of Ranvier, there has been interest in determining whether its location in injured neurons is determined by the axons or the sheath cells themselves.[2]

Cellular Mechanisms of Recovery and Regeneration

The optic nerve axon has a unique structure. Its cell body lies in the retinal ganglion cell layer, and its axon runs centrally for approximately 50 millimeters; one-half its length lies outside the orbit. In the cranial cavity, the optic nerves project to their terminus in the lateral geniculate body. In the last 10 years, it has been shown that a great deal of traffic of chemicals and other cellular components moves between the eye and the brain within the individual axons of the optic nerve.

The movement of materials toward the brain is called anterograde axoplasmic transport; the movement in the reverse direction is called retrograde axoplasmic transport. Although the axons measure only a few microns in diameter, movement in both directions and at various velocities is maintained. The mechanisms that underlie axoplasmic transport are of critical importance because the materials conveyed are probably essential for the regeneration of an injured neuron and because the biochemical signal that the neuron has been injured may be

conveyed by retrograde axoplasmic transport.[16,24] One likely candidate for this injury signal is a calcium-binding protein called calmodulin.[25] Another possible signal that the axon is injured may be the premature return of materials normally conveyed by axoplasmic transport, which are unable to reach their usual destination,[16,26] for example, membrane fragments or neurotransmitters that would normally be removed or modified at the axon terminal. In addition, a protein known as nerve growth factor can be taken up in the terminal region of some neurons and transmitted in retrograde axoplasmic flow.[27] Its absence might also trigger the complex metabolic and morphologic changes that are known as the chromatolytic reaction.[27] The severity of this reaction can lead alternatively to degeneration, survival, or recovery.

Tissue culture of nerve and glial cells has made possible the study of a number of factors involved in neuronal growth and survival. Factors studied have included macromolecular agents, the ionic environment, electric and ionic currents, and the nature of the surface on which the cells are cultured.[28] Various substrates have been used for the growth of chick embryo ciliary ganglion cells, including sulfonated tissue culture plastic containers, collagen-coated containers, and polyornithine coating.[28] Because these substrates have different affinities for various proteins, it has been possible to identify (1) a ciliary neurotrophic factor (CNTF) and (2) a conditioned medium-derived neurite promoting factor (NPF).[28] The CNTF appears in the intraocular tissues that are innervated by ciliary nerves at a specific period of embryonic life and promotes survival of the ciliary neurons.[28] The NPF elicits a lavish growth of ciliary neurites in a radial direction from ciliary ganglion explants. In one study, in the absence of NPF, radial growth of neuritis occurred for only two hours, after which they grew in a circular direction.[29,30] This research suggested that the polyornithine media bound the NPF and defined avenues for neuritic growth.[30] Further experiments will be needed to determine (1) if there are in vivo terrains that do not permit neuritic growth, (2) the types of cells that release NPFs, (3) the temporal aspects of NPF production, and (4) the existence in intracellular spaces of materials capable of binding NPF. An anti-NPF antiserum might prove as useful in defining the role of NPF as anti-nerve growth factor serum has proved in defining the biological role of nerve growth factor.[31]

The molecular requirements for neuronal survival have been studied with chick embryo retinal cells and ciliary ganglion cells in tissue culture using chemically defined media.[30] The development of a serum-free supplement has been an important advance because it allows definition of the chemical environment necessary for cell survival and eliminates extraneous chemicals that can interfere with metabolic studies. Using purified monolayer cultures from the retina, investigators have studied uptake mechanisms for putative neurotransmitters and other substances.[32] Studies of the role of specific chemicals that selectively enhance the growth of certain types of cells, while selectively retarding the growth of other cells, have just begun. The role of the common ions in the neuronal environment also has come under investigation. Potassium ions appear to support neuronal survival in cultures from newborn rat cerebellum and chick embryo ciliary ganglion,[30] whereas calcium ions are important for the rapid phase of axoplasmic transport.[33] In peripheral nerves a calcium ion concentration of 3 to 5 mM promotes the regeneration of the nerve more effectively than does the normal ionic concentration in extracellular fluid, that is, 1.5 to 2 mM;[33] potassium ions are required in a concentration of 4 mM. A concentration of calcium greater than 35 mM blocks axoplasmic transport.[33]

Important tools have been developed to identify the origin of cells that contribute to regenerating tissue. These cell markers and immunological techniques can distinguish populations of cells such as neurons, Schwann cells, fibroblasts, astrocytes, and oligodendroglial cells. One reason that neurons from specific regions of the brain and spinal cord are studied more frequently than those from other regions is that they contain easily identifiable markers such as monoamines. A specific immunochemical marker for retinal ganglion cells is not yet available, but the development of one would be valuable in studies of optic nerve rescue and regeneration.

Another important advance has been the development of tissue culture systems for the study of trophic and neurite-promoting interactions in the retinotectal system. The procedure involves the simultaneous culturing of retinal neurons and neurons from the tectum. Target organ cells seem to be necessary to stimulate the growth of the retinal cells.[34] Higher levels of activity of the enzyme choline acetyltransferase were produced when both kinds of cells were cultured together than when either kind of cell was cultured alone.[34] The optimum ratios of retinal cells to tectal cells and the developmental stages of the cultured cells have been studied.[29] Further definition of the physical and chemical environment necessary for the support of such cultures could be significant.

Molecular Aspects

There are five areas of research at the molecular level in which recent advances have been made that may be significant for rescue and regeneration of the optic nerve: (1) biochemical characterization of the many components within the nerve and glial

cells under conditions of growth, injury, degeneration, and regeneration;[35] (2) biochemistry of the neuron-glia interaction and the interaction between nerve cells and the nerve and muscle cells with which they synapse; (3) role of specific genetically controlled molecules in organizing and guiding both the initial and regenerative development of the nervous system;[36] (4) use of biochemical tags for identifying specific cell types and measuring cell function in degenerating and regenerating neurons; and (5) effect of pharmacological agents on growth, degeneration, rescue, and regeneration.

The detailed biochemical characterization of the components of the nerves and glial cells has just begun. The cytoskeletal components of the axoplasm have been identified in only a preliminary way, and the biochemical basis for axoplasmic transport has not been fully established. Moreover, understanding of the basic mechanisms of axoplasmic transport is incomplete, as are the mechanisms for the production and transport of the membranous organelles. The latter requires the coordinated activity of at least three intracellular systems. The rough endoplasmic reticulum synthesizes the polypeptide backbone of membrane proteins, lysosomal enzymes, and proteins that will be secreted. Proteins are transported by way of the smooth endoplasmic reticulum to the Golgi system, where prosthetic groups such as distal sugars and sulfate are added. These complex molecules usually are discharged intracellularly in the form of vesicles, some of which are incorporated into the plasma membrane. The smooth endoplasmic reticulum within the axon appears to play an important role in the fast component of axoplasmic transport. It may function also in the local synthesis of lipids and may have an important role in the regulation of calcium ions.[33] The vesicles, which are produced in the Golgi apparatus, appear to be important in neural development and regeneration; they seem to contain packets of neurotrophic substances and other substances involved in neurotransmission and maintenance of postsynaptic components.[37] The protein coat of the vesicles recently has been studied using a fluorescent anticlathrin serum to search for the interaction between coated vesicles and neurofilaments.[37]

The precursors of essential phospholipids are poorly incorporated within axons, and it has been proposed that local lipid synthesis is dependent on adjacent cells such as Schwann cells.[38] The enzymatic basis for such activity in ligated nerves has been studied.[38] Other experiments have involved local blockage of synthesis with the drug actinomycin D.[38]

Gangliosides are also important components of the nervous system. The chemical changes in the composition of gangliosides have been investigated primarily in peripheral nerve degeneration and to a limited extent in spinal cord. A specific protein has been found in astrocytes, especially in injured areas in the central nervous system. This protein is called glial-fibrillary acidic protein (GFA), and it is markedly increased in gliotic tissue.[35] The physicochemical characterization of this protein and the mechanisms of assembly and disassembly of its filaments have been under study, especially with regard to the effects of various substances on fibrous gliosis of the retina and optic nerve after a crushing injury.[35]

The biochemistry of the bidirectional interactions between a neuron and the cells with which it connects are being studied in embryonic and early postnatal development, and after disconnection.[16] Not only is the neuron influenced by its target cell, but it in turn exerts an effect on its target cell, for example, development and location of receptor sites for neurotransmitters. In addition, the neuron is influenced by the neurons that synapse upon it and the glial cells in its environment. Within the nervous system, these bidirectional effects are known as transsynaptic regulation.[16] The effects can be both anterograde and retrograde. Transsynaptic retrograde degeneration has been demonstrated in the human visual system. Important biochemical information has been obtained by studying the long-term interdependence of neurons and their peripheral target organs, for example, the relationship between spinal motoneurons and skeletal muscle cells.[39] Cyclic AMP, which is trophically regulated in muscle, seems to be important in the communication between muscle cells and motoneurons. Another group of substances under study are the cell surface macromolecules in the muscles and motoneurons, whose interactions may be responsible for the intercellular recognition process.[40]

The role of specific genetically controlled molecules in organizing and guiding the initial and regenerative development of the nervous system has also been the subject of intensive study. In the Siamese cat, for example, an abnormal projection of optic nerve fibers occurs from the retina to the lateral geniculate nucleus.[36] Unlike the fibers in ordinary domestic cats and other carnivores and primates, the fibers originating from the nasal side of the retina do not completely cross to the opposite side of the brain. This anomaly appears to be the result of an abnormality in the gene that encodes for the structure of tyrosinase, an enzyme responsible for one stage in the biosynthesis of melanin in the retina.[36] Evidence that the abnormality in the retinal projection results from the absence of melanin comes from the appearance of similar abnormalities in various mammalian albino mutants having defects in the genes that encode for other enzymes in the pathway of melanin synthesis.[36]

Chemical agents are thought to play an important role in stimulating axon outgrowth and directing neurons to their appropriate targets. A current candidate is nerve growth factor (NGF), which is

believed to be a prototype of a class of proteins that enhance or direct neuronal growth. NGF promotes optic nerve regeneration in fish and amphibia; a human disorder, familial dysautonomia, in which abnormal development of sympathetic and sensory neurons occurs, is believed to be related to a lack of NGF. Because not all nerves are affected by NGF, analogous agents that enhance directional axon growth in other types of neurons are being sought.

Biochemical methods have been particularly useful for identifying cell types and assessing cellular viability, both of which are essential for demonstrating that regeneration has proceeded successfully from the site of injury to the target organ. Immunochemical markers are being developed to tag specific cell types, and as mentioned previously, monoamines have been used for identifying certain groups of brain cells. Another material known as substance P is an undecapeptide, which functions as a neurotransmitter-like substance for dorsal root afferent fibers that terminate in the dorsal horn of the spinal cord.[41] The uptake of various materials into cells at the proximal or distal end of the axon (for example, horseradish peroxidase and radioactive amino acids) has been used for tracing pathways and demonstrating continuity.[42,43] Labeled precursors of RNA taken into retinal ganglion cells have been studied in the goldfish.[44,45] In addition, similar studies have been directed toward the radiolabeling of axonally transported protein including tubulin and glycoproteins.[46,47]

Immunochemical methods have been used to localize enzymes in the goldfish optic nerve, specifically, the localization of (Na^+, K^+)-ATPase to the regions of the nodes of Ranvier.[48] The enzymes cholinacetyltransferase and acetylcholinesterase also have been studied in goldfish.[49] A toxin obtained from snakes (alpha-bungarotoxin) is useful for identifying receptor sites to which the neurotransmitter acetylcholine binds. Although it is not yet known whether acetylcholine serves as a neurotransmitter in the goldfish (or in higher vertebrates) an increased binding of labeled bungarotoxin in the goldfish tectum after reinnervation following axotomy has been demonstrated.[50] Nerve growth factor has been studied for its effects on acetylcholinesterase and acetylcholine receptors in tissue cultures of chick embryo sympathetic neurons and on a responsive clonal cell line of rat pheochromocytoma.[51]

A number of pharmacological agents affect the structural integrity of neurons and/or glial cells. A number of drugs have been particularly useful for inducing chemical axotomy. For example, injecting 6-hydroxydopamine into the cisterna surrounding the brain of rats destroys the forebrain noradrenergic projection, while there is a regenerative response of noradrenergic fibers in the cerebellum and brainstem.[52–54] Intraventricular injections of 5,6-DHT (dihydroxytryptamine) produce axotomy in the bulbospinal serotonin system.[55,56] In the regeneration that follows, neurotransmitter biosynthetic mechanisms recover extensively and function is restored. This improvement seems to be due to a combination of axonal regeneration, increased utilization of the transmitter, and development of receptor supersensitivity in areas of incomplete regeneration.[55,56] Selective loss of specific neuronal systems can be obtained with the direct application of colchicine, which affects the neurotubular mechanisms.[57] Nonselective injuries also are frequently produced by anoxia or nonspecific poisons.

A variety of drugs appear to have important effects on the functional connections between neurons of the visual system. Some have been tested in experimental amblyopia where the central input from the nonamblyopic eye is overwhelmingly predominant.[58] This predominance may result from inhibition of input from the amblyopic eye. Drugs which diminish the inhibitory actions of neurotransmitters such as the catecholamines or gamma-aminobutyric acid (GABA) have been tested in experimental amblyopia. For example, 6-hydroxydopamine, which destroys catecholamine-containing neurons, prevented loss of binocularity in monocularly deprived kittens, whereas intraventricular injection of norepinephrine increased the loss of binocularity.[58] Another agent, Naloxone, has been studied because one of its effects is to antagonize the inhibitory effects of the central nervous system transmitter, GABA.[58] Naloxone also has been used successfully to improve recovery in cats subjected to cervical spinal trauma.[59] The mechanisms of drug action may be indirect, for Naloxone probably prevents the lowering of systemic blood pressure and improves local spinal cord blood flow.

Conflicting results were reported when GABA was administered to amblyopic cats, substantial restoration of binocular input to the visual cortex was demonstrated by single-unit recordings (see *Volume Two, Part Five, Report of the Strabismus, Amblyopia, and Visual Processing Panel*).

Other drugs that appear to improve the binocularity of monocularly-deprived cats are bicuculine, and intravenous administration of ammonium chloride and ammonium acetate.[58] Exogenous gangliosides have been investigated to determine whether intraocular administration will improve regeneration in the crushed optic nerve of carp fingerlings.[60] The results were negative, although the gangliosides do have a useful effect on synapse formation in tissue culture systems. A specific cyclic nucleotide (N602—dibutyryl adenosine 3′,5′-monophosphate) has been identified; it seems to facilitate the regrowth of interrupted axons by its effect on contractile proteins and microtubules within a regenerating axon stump.[23]

RESEARCH NEEDS AND OPPORTUNITIES

Although much effort has been directed toward the problems of reconnecting a severed optic nerve and reestablishing functional recovery, clinicians and basic scientists have had less interest in applying information on central nervous system regeneration to the rescue of injured neurons in the optic nerve and retina in patients with nonprogressive disorders of these structures. The Society for Neuroscience has established a Section on Regeneration to provide a forum for the increasing number of reports on development, regeneration, and plasticity of the nervous system, but ophthalmologic and other vision research organizations have not followed suit. Therefore, an immediate problem is to focus attention on the many opportunities that exist for studies of rescue and regeneration in the optic nerve and retina.

There is a need for further analysis of systems that regenerate successfully, particularly in teleost fish, which have myelinated optic nerves. The phenomena of axoplasmic transport—initiation, control, and metabolism—need to be defined further. The relationships between events occurring at the synapse or the cut end of the axon and the cell body need to be determined to find ways to enhance axonal regrowth. Little knowledge exists of the effects of neighboring cells and specific molecules on the process of regeneration. The effects of conditioning lesions and previous injuries on neurons that regenerate successfully also require further study. Closely associated with the study of axons that regenerate successfully is the analysis of the relationship between the normal retinal ganglion cell body and its optic nerve axon. This involves not only the transport of molecular building blocks, but very likely chemical signals that may enhance or inhibit the production of these molecules.

Animals in which optic nerve regeneration does not occur need further study, particularly with regard to the interaction between axons and glia. The rescue and regeneration of neurons within the retina have received hardly any attention. Although interest has increased in the pathogenetic mechanisms of the retinal dystrophies and degenerations, little attention has been paid to factors that might promote the survival of nerve cells and prevent the proliferation of non-neuronal elements in the retina.

At the cellular level, tissue culture of retinal neurons and glial cells should be encouraged. Techniques for isolating and maintaining individual types of cells (for example, retinal ganglion cells, bipolar cells, horizontal cells, photoreceptors, and Müller cells) will have to be developed (see Chapter 8, "Retinal Pigment Epithelium;" Chapter 9, "Photoreceptors, Visual Pigments, and Phototransduction;" Chapter 10, "Retinal Organization, Neurotransmission, and Adaptation" and Chapter 11, "Glial Cells and the Retinal Microenvironment"). As these become available, it may be possible to define cellular interactions and the conditions that optimize growth and regeneration of such cells. Tissue culture techniques also may be useful for initially screening the efficacy of therapeutic agents, although in vivo studies would be needed at a later stage.

RECOMMENDATIONS

Based on the foregoing assessment of recent accomplishments, current activities, and research needs and opportunities in "Rescue and Regeneration of Neurons in the Optic Nerve and Retina," the Panel has made the following recommendations concerning research in this subprogram over the next five years. These have all been designated as Program Development Priorities and include areas of ongoing research in which new knowledge and techniques offer particular opportunities for scientific progress, or promising new areas of research in which there is little or no support at present but where there is both great need and high potential for success. Such areas are judged to warrant significantly increased support over the next five years, provided that high quality applications for research grants in these areas are forthcoming.

Program Development Priorities

- Develop new primate and other adult mammalian models for research on optic nerve and retinal rescue and regeneration.
- Investigate neuronal-glial interactions in the retina and optic nerve and their relationship to the problem of regeneration.
- Expand research on biochemical and physiological processes in the retina and optic nerve to elucidate the factors which prevent degeneration and enable regeneration.

RESOURCE REQUIREMENTS

After reviewing current research grant support in each of these categories and assessing the need and potential for future development, the Panel has estimated the number of projects it believes are

needed to carry out its recommendations in FY 1983. These estimates are shown in the table on the following page. For a discussion of the general basis and significance of these projections, see the "Summary" at the beginning of this report.

RESOURCE TABLE

RESCUE AND REGENERATION OF NEURONS IN THE OPTIC NERVE AND RETINA

	No. of Grants FY 1981	Panel Recommendation FY 83	
		Add. Grants	Total Grants
Program Development Priorities			
A. Develop new primate and other adult mammalian models of optic nerve and retinal rescue and regeneration.	1	2	3
B. Investigate neuronal-glial interactions in the retina and optic nerve and their relationship to the problem of regeneration.	0	2	2
C. Expand research on biochemical and physiological processes in the retina and optic nerve to elucidate factors which prevent degeneration and enable regeneration.	1	1	2
Subtotal Grants	**2**	**5**	**7**
(% of Program)	**(1)**	**(5)**	**(1)**
Total Estimated Cost	**$136,000**	**$599,000**	**$735,000**

REFERENCES

1. Aquayo AJ, et al: Ensheathment and myelination of regenerating PNS fibers by transplanted nerve glia. *Neurosci Lett* 9:97-104, 1978.
2. Bray GA, et al: Interactions between axons and their sheath cells. *Annu Rev Neurosci* 4:127-162, 1981.
3. *Report of the Panel on Stroke, Trauma, Regeneration and Neoplasms to the National Advisory Neurological and Communicative Disorders and Stroke Council.* US DHHS Pub No (NIH) 79-1915, 1979.
4. Monti Graziadei GA, et al: Reinnervation of the olfactory bulb after section of the olfactory nerve in monkey (*Saimiri sciureus*). *Brain Res* 189:343-354, 1980.
5. Sharma SC: The effect of bilateral partial tectal ablation on optic nerve innervation in adult goldfish. *Brain Res* 183:453-457, 1980.
6. Springer AD: Conversion of a spontaneous to an induced ipsilateral retinotectal projection in goldfish. *Brain Res* 193:254-257, 1980.
7. Grafstein B, McQuarrie IG: Role of the nerve cell body in axonal regeneration, in Cotman CW (ed): *Neuronal Plasticity.* New York, Raven Press, 1978, pp 155-195.
8. Landreth GE, Agranoff BW: Explant culture of adult goldfish retina: Effect of prior optic nerve crush. *Brain Res* 118:299-303, 1976.
9. Lanners HN, Grafstein B: Effect of a conditioning lesion on regeneration of goldfish optic axons: Ultrastructural evidence of enhanced outgrowth and pinocytosis. *Brain Res* 196:547-553, 1980
10. McQuarrie IG, Grafstein B: Effect of a conditioning lesion on optic nerve regeneration in goldfish. *Brain Res* 216(2):253-264, 1981.
11. Springer AD, Agranoff BW: Effect of temperature on rate of goldfish optic nerve regeneration: A radioautographic and behavioral study. *Brain Res* 128:405-415, 1977.
12. Richardson PM, et al: Axons from CNS neurones regenerate into PNS grafts. *Nature* 284:264-265, 1980.
13. Aquayo AJ, et al: Rat Schwann cells cultured in vitro can ensheath axons regenerating in mouse nerves. *Neurology (Minneapolis)* 29:589, 1979.
14. Kao CC, et al: The mechanism of spinal cord cavitation following spinal cord transsection: Electron microscopic observations. *J Neurosurg* 46:745-756, 1977.
15. Marx JL: Regeneration in the central nervous system. *Science* 209:378-380, 1980.
16. Veraa RP, Grafstein B: Cellular mechanisms for recovery from nervous system injury: A conference report. *Exp Neurol* 71:6-75, 1981.
17. Cotman CW, Banker GA: The making of a synapse. *Rev Neurosci* 1:1-62, 1974.
18. Raisman G, Field PM: A quantitative investigation of the development of collateral reinnervation after partial deafferentation of the septal nuclei. *Brain Res* 50:241-264, 1973.
19. Geldred JB: Quantitative anatomical and behavioral analyses of regeneration and collateral sprouting following spinal cord transsection in the nurse shark (*Ginglymostoma cirratum*). *Acta Neurobiol Exp (Warsz)* 39:121-142, 1979.
20. Rosen JM, et al: Suture and sutureless methods of repairing experimental nerve injuries, in Jewett DL, McCarroll HR (eds): *Nerve Repair and Regeneration.* St Louis, CV Mosby Co, 1980, pp 235-242.
21. Soffer D, Raine CS: Morphologic analysis of axoglial membrane specialization in the demyelinated central nervous system. *Brain Res* 186:301-313, 1980.
22. McIlwain DL, Farel PB: Initiation and time course of mitosis of non-neuronal cells after spinal motoneuron axotomy. *Brain Res* 178:519-528, 1979.
23. *Spinal Cord Regeneration.* U.S. House of Representatives, Committee on Interstate and Foreign Commerce, Subcommittee on Health and the Environment, Serial No 96-181, 1980, p 248.
24. Grafstein B: Axonal transport: The intracellular traffic of the neuron, in Kandel ER (ed): Cellular Biology of Neurons. *Handbook of Physiology.* Bethesda, American Physiological Society, 1977, vol I.
25. Bray D, Gilbert D: Cytoskeletal elements in neurons. *Annu Rev Neurosci* 4:505-523, 1981.
26. Bisby MA, Bulger VT: Reversal of axonal transport at a nerve crush. *J Neurochem* 29:313-320, 1977.
27. Grafstein B, Forman DS: Intracellular transport in neurons. *Physiol Rev* 4:1168-1283, 1980.
28. Varon S, Adler R: Nerve growth factors and control of nerve growth. *Curr Top Dev Biol* 16:207-252, 1980.
29. Adler R: Trophic and neurite-promoting factors in eye development, in Hollyfield JG (ed): *The Structure of the Eye.* New York, Elsevier-North Holland, to be published.
30. Varon S, Adler R: Trophic and specifying factors directed to neuronal cells. *Adv Cell Neurobiol* 2:115-163, 1981.
31. Turner JE, Delaney RK: Retinal ganglion cell response to axotomy and nerve growth factor antiserum in the regenerating visual system of the newt (*Notophthalmus viridescens*): An ultrastructural morphometric analysis. *Brain Res* 177:35-47, 1979.
32. Hyndman A, Adler R: Effects of monosodium glutamate on purified monolayers of chick embryo retina neurons and non-neuronal cells. *Invest Ophthalmol Vis Sci* (suppl) 20(3):80, 1981.
33. Ochs S: Calcium requirement for axoplasmic transport and the role of the perineurial sheath, in Jewet DL, McCarroll HR (eds): *Nerve Repair and Regeneration.* St Louis, CV Mosby Co, 1980, pp 77-87.
34. Hyndman AG, Adler R: Neural retinal development in vitro: Effects of tissue extracts on cell survival and neurite development in purified neuronal cultures. *Dev Neurosci,* to be published.
35. *Spinal Cord Regeneration.* U.S. House of Representatives, Committee on Interstate and Foreign Commerce, Subcommittee on Health and the Environment, Serial No 96-181, 1980, p 238.
36. Stent GS: Strength and weakness of the genetic approach to the development of the nervous system. *Annu Rev Neurosci* 4:163-194, 1981.

37. *Spinal Cord Regeneration*. U.S. House of Representatives, Committee on Interstate and Foreign Commerce, Subcommittee on Health and the Environment, Serial No 96–181, 1980, p 251.
38. *Spinal Cord Regeneration*. U.S. House of Representatives, Committee on Interstate and Foreign Commerce, Subcommittee on Health and the Environment, Serial No 96–181, 1980, p 233.
39. *Spinal Cord Regeneration*. U.S. House of Representatives, Committee on Interstate and Foreign Commerce, Subcommittee on Health and the Environment, Serial No 96–181, 1980, p 205.
40. *Spinal Cord Regeneration*. U.S. House of Representatives, Committee on Interstate and Foreign Commerce, Subcommittee on Health and the Environment, Serial No 96–181, 1980, p 227.
41. Tessler A, et al: Recovery of substance P in the cat spinal cord after unilateral lumbosacral deafferentation. *Brain Res* 191:459–470, 1980.
42. Lavail JH, Lavail MM: The retrograde intraaxonal transport of horseradish peroxidase in the chick visual system: A light and electron microscopic study. *J Comp Neurol* 157:303–358, 1974.
43. Maxwell JK, Elam JS: Amino acid incorporation into proteins of degenerating and regenerating goldfish optic tracts. *Exp Neurol* 67:118–130, 1980.
44. Burrell HR, et al: RNA metabolism in the goldfish retina during optic nerve regeneration. *J Neurochem* 31:289–298, 1978.
45. Murray M: ^{3}H-uridine incorporation by regenerating retinal ganglion cells of goldfish. *Exp Neurol* 39:489–497, 1973.
46. *Spinal Cord Regeneration*. U.S. House of Representatives, Committee on Interstate and Foreign Commerce, Subcommittee on Health and the Environment, Serial No 96–181, 1980, p 237.
47. Burrell HR, et al: Increased tubulin messenger RNA in the goldfish retina during optic nerve regeneration. *Brain Res* 168:628–632, 1979.
48. Schwartz M, et al: Immunocytochemical localization of (Na^+,K^+)-ATPase in the goldfish optic nerve. *J Neurochem* 36:107–115, 1981.
49. Francis A, Schechter N: Activity of choline acetyltransferase and acetylcholinesterase in the goldfish optic tectum after disconnection. *Neurochem Res* 4:547–556, 1979.
50. Schwartz M, et al: Histological localization of binding sites of alpha-bungarotoxin and of antibodies specific to acetylcholine receptor in goldfish optic nerve and tectum. *Brain Res* 194:171–180, 1980.
51. *Spinal Cord Regeneration*. U.S. House of Representatives, Committee on Interstate and Foreign Commerce, Subcommittee on Health and the Environment, Serial No 96–181, 1980, p 193.
52. Schmidt RH, Bhatnager RK: Intracisternal dose-response analysis of 6-hydroxydopamine induced noradrenergic sprouting in the neonatal rat cerebellum. *J Pharmacol Exp Ther* 212:456–461, 1980.
53. Schmidt RH, et al: Regenerative critical periods for locus coeruleus in post-natal rat pups following intracisternal 6-hydroxydopamine: A model of noradrenergic development. *Brain Res* 191:173–190, 1980.
54. *Spinal Cord Regeneration*. U.S. House of Representatives, Committee on Interstate and Foreign Commerce, Subcommittee on Health and the Environment, Serial No 96–181, 1980, p 214.
55. Bjorklund A, Wiklund L: Mechanisms of regrowth of the bulbospinal serotonin system following 5,6-dihydroxytryptamine induced axotomy: I. Biochemical correlates. *Brain Res* 191:109–127, 1980.
56. Wiklund L, Bjorklund A: Mechanisms of regrowth of the bulbospinal serotonin system following 5,6-dihydroxytryptamine induced axotomy: II. Fluorescence histochemical observations. *Brain Res* 191:129–160, 1980.
57. Karlsson JO, et al: Effect of colchicine on axonal transport and morphology of retinal ganglion cells. *Z Zellforsch* 115:265–283, 1971.
58. Duffy FH, Burchfiel JL, Snodgrass SR: The pharmacology of amblyopia. *Ophthalmology* 85(5):489–495, 1978.
59. Faden AI, et al: Opiate antagonist improves neurologic recovery after spinal injury. *Science* 211:493–494, 1981.
60. Beuttler H, et al: Effect of exogenous gangliosides on nerve cell regeneration. *Jpn J Exp Med* 50:63–65, 1980.

RELATED AREAS OF RESEARCH OPPORTUNITY AND NEED

13

NONINVASIVE TECHNIQUES IN THE STUDY OF RETINAL DISORDERS

INTRODUCTION

BECAUSE THE RETINA is well protected by the vascular and tough scleral coats of the eye, it is not easily accessible for study. Biopsy of retinal tissue is rarely performed because of the delicacy of the tissue and the inherent risks to vision involved. Thus, to perform more accurate diagnoses and acquire a greater understanding of retinal disease processes, it is essential to utilize noninvasive test procedures, that is, to deduce the necessary information through the judicious use of light or sound waves or by other means that do not require penetrating the globe. The development of these noninvasive methods has become a fruitful and exciting investigative area.

Improved noninvasive tests may lead to a more accurate classification of retinal disease processes, improved ability to differentiate among retinal anomalies, and new or improved methods of treating retinal diseases. The principal objectives of this research are to define accurately the natural history of retinal disorders and identify functionally abnormal cells. Such studies attempt to answer a number of important questions: What are the first signs of the anomaly? Does it spread? What other tissues are involved? Is it an acute or a chronic process? Is there still capacity for recovery at certain stages of the disease? Is the lesion defined by the anomaly observed ophthalmoscopically, or are there nonvisible functional alterations that extend beyond the observed boundaries? Does one test provide the same information as that obtained by other methods?

The precise localization of the retinal defect produced by pathological processes is crucial in determining target loci for treatment and evaluating the status of the disease. Tests of both localized and mass responses help in determining whether a given therapy is successful. A detailed picture of the pathological process is also necessary for developing suitable animal models of human retinal disease.

Fine distinctions also need to be made between inherited congenital anomalies and those acquired in utero or shortly after birth. Some distinctions can be made if carefully documented family trees can be established, but this is not always possible. Refined diagnostic procedures that enable early detection of affected individuals or carriers are necessary for sound genetic counseling.

Because improved methods for early diagnosis and more precise localization of retinal disorders are central to the realization of the goals of the National Eye Institute's Retinal and Choroidal Diseases program, the Institute and the National Advisory Eye Council have strongly emphasized the need for more research in this area. [1,2]

SUBPROGRAM OBJECTIVES

- To develop improved noninvasive tests of retinal function.
- To apply noninvasive techniques to improve the prevention, diagnosis, and management of retinal disease in human infants and adults.

OVERVIEW OF CURRENT RESEARCH SUPPORT

Noninvasive testing and methods development represents a cross-cutting area of research which is important to all subprograms of the Retinal and Choroidal Diseases program. In FY 1981 the National Eye Institute supported 10 grants in this subprogram at a total cost of $1,176,000 for research on noninvasive tests of retinal neural function and blood flow, and for the development and application of television ophthalmoscopy. Twenty-four other NEI grants, classified in other Retinal and Choroidal Diseases subprograms, included research involving noninvasive techniques for assessing the retina and choroid. The specific research problems, needs, and opportunities relating to the use of these techniques are detailed in Chapter 1, "Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities;" Chapter 3, "Tumors;" Chapter 4, "Developmental and Hereditary Disorders;" Chapter 5, "Macular Degeneration;" Chapter 8, "Retinal Pigment Epithelium;" and Chapter 10, "Retinal Organization, Neurotransmission, and Adaptation."

RECENT ACCOMPLISHMENTS

Ophthalmoscopic Techniques

Ophthalmoscopic devices use the optics of the patient's eye to aid the clinician in viewing the internal structure, including the retina. When coupled to photographic elements, these instruments are called fundus cameras. After years of little development in this area, a broad range of new instruments has appeared recently; these allow wide-angle photography of the retina and offer considerable advantage to the diagnostician. One such unit images virtually the entire retina on a single photographic frame;[3] others make possible views of 45 degrees or more. Anyone who has patiently pieced together photomontages of several retinal photographs can attest to the significance of these developments.

Infrared fundus cameras also are now available which permit the investigator to align and focus the subject's eye in a room with reduced illumination and to photograph the retina without dilating the pupil. The infrared light, invisible to the patient, is converted into visible light for the investigator to see necessary details of the retina on a TV-like display.

Infrared cameras also make possible a major new diagnostic approach, the linking of fundus cameras to perimetric testing. A perimeter is an instrument designed to evaluate visual function at topographically defined loci in the visual field, that portion of the external environment of the observer in which the steadily fixating eye(s) can detect visual stimuli.[4] The examiner can look at a retinal lesion on the TV screen and test visual function by back-projecting a target through the camera to the appropriate retinal area. In this way, a visible retinal lesion can be related to the degree of functional involvement.[5–10]

Holographic techniques can be applied to fundus photography to obtain three-dimensional views in a single interferogramic record. Sequential recordings allow detection of very fine changes over time. Early successful results have now been published,[11–13] although there is a problem with the amount of light needed for recording the hologram.

By using fundus cameras modified for special purposes, fine details of fundus changes have been revealed. Physical changes in the contour of the cup-like optic nerve head have been found to be temporally related to the development of visual field defects in glaucoma. New methods for detecting such changes have been developed using both superposition and stereoscopic techniques, which enable comparison of near-identical fundus pictures of the optic nerve head photographed at different times.[14]

Selective filtering, for example, using red-free wavelengths, has permitted detection of early retinal nerve fiber bundle anomalies.[15] Measuring the reflected spectrum from the retina following selective bleaching of the light-sensitive visual pigments contained in the photoreceptors allows detection of the individual photosensitive pigments and measurements of their rates of bleaching and reformation. This important index of photoreceptor response capability[10,16] has been useful in detecting disease processes that affect specific receptor groups (for example, rods versus cones or one group of cones versus another).[17] Collectively, such tests are termed fundus reflectometry. Similar techniques also have been used to measure blood flow, oximetry, and permeability of retinal and optic nerve head capillaries.[18,19]

Ultrasonographic Techniques

Ultrasonography uses the physical properties of high-pitched sound waves traveling through a liquid medium and reflected from interposed structures to locate foreign bodies in the orbit, look behind opacities for retinal detachments and tumors, and measure ocular dimensions. Techniques of measurement, data analysis, and display formats for ultrasonography have evolved rapidly in recent years.[20,21]

One recent application has been the use of ultrasound, coupled with minicomputers, to provide read-outs for the ophthalmic surgeon to use in selecting intraocular lens implants of an appropriate power for individual aphakic patients. Recently, ultrasound has been used therapeutically to attempt to break vitreous membranes and as a possible approach to tumor treatment. [22,23]

Other Noninvasive Methods

Visual functions can now be tested noninvasively in a variety of ways, either objectively or subjectively. Examples of the former are fundus reflectometry (discussed above), light-evoked ocular potentials, and contraction of the pupil following exposure to controlled light stimuli. Subjective responses require active participation by the individual being examined. The subject may simply need to indicate when a target is first seen or may be required to choose one of two targets as being brighter, redder, nearer, larger, or moving more rapidly. Such subjective tests are generally called psychophysical tests of vision.

One psychophysical test in which substantive progress has been made is perimetry. In the years following World War II, well-calibrated perimeters were developed; they used higher background adapting levels and were capable of kinetic (detection of a moving target) and static (detection of a nonmoving target of variable luminance) measurements. These refined instruments set the stage for the rapid advances being made today. Perimetry is still an art, although investigators have moved rapidly toward defining its scientific base. Semiautomated and automated devices have been developed to perform examinations; to monitor eye movements and responses, and to follow a variety of preprogrammed schemes for testing various features of the visual field. Others are essentially screening devices for given target populations. Many papers on this topic were presented at a meeting of the International Perimetric Society in Bristol in 1980. [24]

A second major development is the evolution of the fundus perimeter mentioned previously in this chapter. This is a combination of the infrared fundus camera and the perimeter, [5-9] whose purpose is to allow the examiner to perform a series of functional tests in and around the area of a visualized lesion. More recently, computerized field testing has added a new degree of sophistication to visual field examination.

By combining a variety of electrophysiological tests, fundus reflectometry, and psychophysical tests, advanced analyses can be performed. [25] Evidence is accumulating that this approach has great value in correlating functional changes with visible abnormalities.

A number of subjective and objective tests have been developed, which have been used to improve localization of lesions at selected points along the visual pathway. The studies have utilized the local (or focal) and retina-wide electroretinogram, [10,26-28] tests of photoreceptor alignment and sensitivity, [9,29,30] dark adaptation, [10,28] increment threshold measurements, [9,29,30] analysis of receptive-field properties, [9,29,30] evaluation of color vision data processing, [31-34] a number of time-dependent measures for lesions central to the optic nerve head, and the visually evoked response. [9,29,30,35,36]

Through the use of such tests, it has been possible to identify in some instances the cellular elements that are the primary targets for the pathological condition, to differentiate subclasses of pathology, and to chart the spread and remission of pathological processes, both within and across the retina. In addition, the knowledge gained from studies of individual disease processes has permitted differentiation among multiple pathological processes and evaluation of the efficacy of some treatments. These tests have been applied to a broad range of retinal diseases, including different types of retinitis pigmentosa, [26,27] stationary night blindness (fundus albipunctatus and Oguchi's disease), [10,16,26] some retinal tumors, central serous choroidopathy, [37] Best's vitelliform disease, [38] involutional macular degenerations, [9,39,40] diabetic retinopathy, retinal detachments, [38] retinal traumas, [41] tractional lesions of the retina, [41,42] glaucoma, anterior ischemic optic neuropathy, other forms of optic neuritis [9,43] including that induced by multiple sclerosis, [35,36,44] and a variety of inflammatory, space-occupying, and ischemic and/or infarct-type lesions of the optic pathways. [45] The fact that this list is growing rapidly and fine discriminations are being made in response to carefully formulated scientific questions is promising for further developments.

A new test that is attracting much attention is the contrast sensitivity function, a version of the modulation transfer function which has had broad acceptance in electronics and optics. Recognition and interpretation of objects may be considered a complex act, dependent upon the synthesis of inputs from objects of different sizes, contrast, brightness, and image sharpness. Each object may comprise a complex of elements with different dimensions (spatial frequencies) and varying contrasts. Thus, a more complete view of an individual's visual capability may be obtained by evaluating just detectable contrast sensitivity to a number of objects of different size. This is somewhat similar to a Fourier analysis of the visual system. Comparable temporal frequency (time resolution) responses as a function of contrast can be assessed as well.

This approach is popular among investigators and clinicians. Available tests have been applied to a broad range of conditions, including aphakia, [45,46]

several retinal diseases,[47,48] glaucoma,[49,50] myopia,[46,51] amblyopia, and retrobulbar optic nerve lesions.[52] However, many published studies lack appropriate controls for such factors as retinal image blur, luminance, pupil size, and test distance. For example, several papers at the International Congress of Ophthalmology and associated symposia held in Japan in 1978 suggested that people who are aphakic have a loss in low-frequency response, that is, are less sensitive to larger dimension size wave patterns. Since then, certain of these findings have been shown to be a magnification artifact.[46] However, studies have revealed true low-frequency fall-off in aged individuals,[53] and also low-frequency changes in patients with open-angle glaucoma.[49,50]

One of the problems in measuring low-frequency responses is that several cycles of the test pattern are needed, and this may extend the test pattern beyond the macular area. Thus, it is not always clear that central visual function is being measured in these instances. The latter problem is important in considering diseases affecting the macula or in measuring early visual function in infants. There has been an excellent suggestion to establish standards for contrast sensitivity testing.[53] Such an effort would help clarify what is being tested and establish meaningful test criteria.

Another important research area is the development of visual function in newborn infants. Studies of sensory deprivation amblyopia have shown that unresolved deficiencies present during one or more critical periods in the development of vision in early life result in profound and largely irreversible visual loss.[54,55] Proper tests of visual function are needed to assess accurately the status of infant vision and evaluate attempts to resolve visual anomalies of infancy. Preferential looking,[56–59] visually evoked responses,[60,61] optokinetic nystagmus, and other techniques are being developed to assess visual acuity, contrast sensitivity functions,[62] color vision,[63] and stereopsis.[64,65] It is hoped that a battery of techniques suitable for clinical application will emerge. Priorities for these studies are presented in *Volume Two, Part Five, Report of the Strabismus, Amblyopia, and Visual Processing Panel.*

Earlier sections of this report have stressed the unique physical, chemical, and functional properties of the visual cell. In particular, it has been known since the last century that the photoreceptors are photomotile in some species such as amphibians and several types of fish. That is, the photoreceptors elongate and contract at different light levels. These actions occur when light level is changed and allow switching of dominant functioning receptor sets (rods and cones) in these species.

Human photoreceptors may also show some degree of phototropism. About a decade ago, it was demonstrated that the photoreceptors pointed toward an anterior point in the eye,[66] and through the use of the Stiles-Crawford function (that is, assessing the directional sensitivity of the retina), it was noted that the reference for alignment approximates the center of the exit pupil.[67] Further, this arrangement can be upset experimentally.[68] Prolonged occlusion of the eye caused alignment dispersal, and wearing a displaced aperture contact lens caused a change in alignment.[69,70] Moreover, in a patient who had a displaced pupil, it was possible to alter a displaced Stiles-Crawford function toward a more normal alignment by having the individual wear a centered aperture contact lens.[68,71]

By following changes in photoreceptor alignment in neurosensory retinal detachment and retinal pigment epithelial detachment, investigators have shown that the retinal pigment epithelium plays a role in maintaining proper alignment of the photoreceptors and associated anatomical elements[72] (see Chapter 8). Further, it is well known that improved photoreceptor alignment often occurs after neurosensory retinal detachments resolve.[38] Determining the status and nature of the mechanisms underlying photoreceptor alignment is important in defining the integrity of the photoreceptor in disease and should be of continuing value in localizing pathological processes. The ability to alter alignment may provide one more means of influencing retinal function positively.[69–71]

A continuing problem for the ocular surgeon in diagnosis is the assessment of visual function in patients who have opacities or scattering bodies in the preretinal ocular media. Several promising leads for this problem are being pursued, including evaluation of interferometric techniques,[73,74] measurements through the sclera (or lid) of the visually evoked potential, and studies of hyperacuity paradigms.

The earliest diagnostic applications of electroretinographic testing (ERG) took advantage of the fact that this easily recorded electrical potential represented a massed discharge from the entire sensory retina and used psychophysical data to separate stimulus conditions for rod and cone activity.[75] Differences between the two photoreceptor types with regard to retinal adaptation, temporal resolution (flicker alternation rates), and spectral sensitivity, allowed independent assessment of the rod and cone systems. For example, only cone-mediated responses are elicited by flashes presented to the light-adapted eye or by rapidly flickering the light at rates higher than 25/sec; rod-mediated activity can be sampled by dim blue flashes of light presented to a dark adapted eye. This testing had immediate application in evaluating infants and children with visual loss for whom extensive psychophysical determinations are not possible.[76]

Though the ERG has provided valuable diagnostic information about many types of eye diseases, its unique properties are well-demonstrated in a class of

conditions known as the congenital cone dysfunction syndromes.[77] Patients with one form of the disease, rod monochromatism, have the following symptoms: nystagmus, poor visual acuity, photophobia, and complete loss of color vision. These symptoms, which are present at birth and continue throughout life, derive from a generalized loss of functioning cones. Although the symptoms also occur in several other eye diseases, such as generalized retinal degeneration, localized macular degeneration, optic nerve disease, or incomplete albinism, a definitive diagnosis can be made quickly using the ERG. Rod monochromats uniquely cannot generate an ERG following response to a bright flickering flash but do show normal dark-adapted (rod) potentials.

Refinements of stimuli have brought electroretinographic analysis of rod and cone function to a highly quantitative level. If a pair of scotopically balanced long and short wavelength flashes are presented at different levels of retinal light adaptation, the functional status of rod and cone activity (as judged by their b-wave amplitudes) can be ascertained.[78]

The time-to-peak of the ERG b-wave (implicit time) following a flash is also of diagnostic value. Many acquired retinal diseases may resemble, either functionally or ophthalmoscopically, the more severe heredoretinal degenerations. Rubella retinitis, for example, or any of the other varieties of chorioretinitis may be difficult to separate from the more severe retinal abiotrophies. When such diseases occur in children on whom reliable subjective testing cannot be performed, ERG testing may be the only means to obtain meaningful data. It has been found that most forms of primary retinal degenerations are associated with an increase in the peak time of rod and cone b-waves of the ERG. Inflammatory diseases of the retina, or localized atrophic changes, on the other hand, produce decrements in ERG amplitude but a normal time-to-peak interval.[79] To measure ERG latency values accurately, techniques were developed to ensure homogeneous distribution of flash luminance across the entire retinal surface.[80,81]

ERG b-wave implicit times also have been of value in distinguishing among forms of retinal degeneration that have different modes of genetic transmission. It was found, for example, that patients with dominantly inherited retinitis pigmentosa can be divided into two subgroups. One group (nominally from pedigrees having incomplete penetrance of the gene) manifest longer photopic b-wave implicit times, whereas patients from pedigrees having a more completely penetrant form of transmission have normal photopic b-wave implicit times.[82] The significance of the implicit time measurements relative to the underlying pathogenetic differences is not known. It would be of interest to determine, for example, whether the increased time required for maximization of the b-wave potential in the incompletely penetrant form of retinitis pigmentosa represents a specific defect in membrane structure or function.

The power of ERG testing as a diagnostic tool is greatly enhanced when components of the complex ERG waveform can be related to specific generating structures in the retina. Changes in ERG responses in ocular disease can then not only aid in defining the locus of the disease within the retinal layers but also provide clues to the nature of the defect itself. The necessary information for this approach comes from a layer-by-layer analysis of those cells in the animal retina that produce the ERG. Over many years, various techniques have been applied to fractionating the ERG into its component potentials. These have included inducing anoxia in specific retinal layers, application of chemical substances that selectively inhibit synaptic activity, and microelectrode penetration of each retinal neuron participating in visual excitatory processes.

Knowledge of which groups of cells contribute to particular portions of the clinical ERG has been particularly helpful in analyzing the several kinds of congenital stationary night-blinding diseases. Patients with one form of night blindness have normal appearing fundi, no rod segment in the dark adaptation curve, and little evidence of a b-wave in their ERG responses. Even more surprising is that fundus reflectometric data from these patients have shown that normal amounts of rhodopsin are present in the rods, and after being bleached this pigment returns in normal fashion.[83] The ERG confirms that the photoreceptors are indeed normal and that it is the postreceptoral activity that is abnormal. Previous ideas about this form of night blindness assumed some sort of rod malfunction or disorder of rhodopsin synthesis; the new data exclude such explanations. The heritable defect, as indicated by the ERG findings, appears rather to lie postreceptorally and to act in some unknown manner to prevent b-wave generation. Each of the other forms of night blindness has proved to be as interesting in expanding basic understanding of adaptation mechanisms, and in each form ERG recordings have been valuable in directing attention to the possible site of the lesion.

Some emphasis recently has been placed on exploring dynamic aspects of the ERG by presenting a sequence of graded flashes and recording a voltage versus intensity function. The slope of the linear portion of this function represents a measure of system "gain" in that it provides an estimate of changing voltage generated by a unit increase of absorbed photic energy. In female carriers of X-linked retinitis pigmentosa, the decreased slope of

the basic curve indicates that this property of retinal electrical function is abnormal.[84]

The existence of fast oscillatory wavelets riding on the ascending limb of the developing b-wave (giving it a notched appearance) has been recognized for many years. These fast frequency components can be easily isolated from the slower ERG potentials by electrically filtering out all frequencies below 60 Hz. The stimulus conditions required to produce the oscillatory potentials reliably in humans also have been carefully worked out.[85] Results from early studies suggested that certain oscillatory potential characteristics could be used as diagnostic aids in various kinds of retinal diseases.[86] In general, diseases of the inner retinal layers were associated with markedly attenuated oscillatory potentials; this finding agreed with the results of a study suggesting that oscillatory potentials were probably generated in the inner plexiform layer.[87] However, many exceptions to this clinical finding were also reported, which made reliable diagnosis based on oscillatory potentials unsatisfactory. For example, results purporting to show unmistakable diminution of oscillatory potential amplitudes in early diabetic retinopathy[88] was not substantiated by other studies.[89]

More recently, the source of the oscillatory potentials was reinvestigated in animals using depth analysis microelectrode recordings.[90] The results indicated that the oscillatory potentials cannot be generated as proximally as was once thought but may in fact derive from the newly discovered interplexiform cells. Clearly, more work is needed to establish the source of these potentials.

A standing potential exists across the eyeball, the amplitude of which varies with retinal adaptation. Because it is difficult to measure the slow DC changes that take place in the eye, advantage is taken of the transient electrical changes that occur when the eye executes saccades between two fixed electrodes. During a prolonged period of dark adaptation, the saccade voltages decrease, reaching a minimum in about six or seven minutes, and they rise slowly to a maximum when the eye is then exposed to a steady bright light. The normal light peak/dark trough ratio is about 2.0.[91] Generalized diseases of the retina markedly decrease this value.

Several lines of research implicate two possible sources of activity that contribute to the electrooculographic (EOG) light rise: the c-wave, which is associated with retinal pigment cell activity[92] and the so-called "light peak," which slowly builds to a maximum after several minutes in the presence of a steady light.[93] Unlike the c-wave, which is generated in response to a K^+ decrease in the subretinal space, the mechanism for generating light peak is not yet fully understood.[94] The c-wave, light peak, and clinically recorded EOG changes are of particular interest in vitelliform macular degeneration (Best's disease), in which there is a markedly decreased EOG light rise but normal ERG a-wave and b-wave amplitudes.[72,95] However, c-wave development in patients with Best's disease has not been studied with DC-ERG techniques. Though difficult to perform because no eye movement or eye blink can be permitted to occur during the several seconds of recording time, measuring c-wave activity would be of great interest in such patients. The disease itself appears localized to the macular region, yet the decreased EOG light rise is more indicative of a generalized retinopathy. Examining some of the slower ERG waves might therefore help delineate the underlying pathogenetic retinal changes.

Although there are obvious advantages to presenting large homogeneous fields of light to the retina (for example, for measuring ERG latencies), other circumstances require that the examiner attempt to record electrical activity from a specific retinal locus. Obvious applications for such stimulation are the many diseases localized to the macular region. If the stimulus is made small and dim (to prevent stray light from exciting peripheral retinal areas), the problem becomes one of detecting a very small signal relative to the normal background noise. For the most part, electronic signal averaging has solved the signal/noise obstacle but not the problem of patient fixation. The question is: How sure is the examiner that the patient is fixating the small stimulus light and not some point to one side of it? Normal subjects can be trained to fixate accurately, and excellent foveal ERGs can be obtained,[96] but for untrained subjects, the problem is more difficult.

One approach is to use a modified direct viewing ophthalmoscope, which projects (under the examiner's control) a small flickering light onto a particular fundal area. To lessen the effects of stray light, a larger steady background surrounds the stimulus spot.[97] Several studies performed with this apparatus show that it has wide application, especially in retinal degenerations in which only electrical responses to luminance changes are relevant.[98]

A second approach is to use a type of stimulus for local ERG recording that uses a reversing grating or checkerboard pattern for the patient to fixate.[99] Although the examiner has less control over exactly which part of the target is being fixated, this technique offers more flexibility in the type of retinal stimulation that can be given. The target itself interchanges light and dark areas in a manner that does not change its overall luminance. If the rate of alternation is above the temporal resolving power of the rod mechanism, peripheral retinal areas "see" only a steady light; the central area on which the target is imaged responds electrically to the local luminance changes produced when the darker areas flip to lighter ones, and vice versa.

Reliable ERGs have been recorded with targets encompassing areas as small as 2 degrees.[100]

Using targets whose spatial properties can be varied has provided new facts about ERG activity. At least two studies indicate that patients with amblyopia have reduced foveal ERGs when pattern stimulation is used.[101,102] This finding implies that under certain circumstances the ERG may reflect properties of retinal spatial organization. Independent evidence from animal studies confirms this idea. For example, when a perfused cat eye cup was stimulated with dot patterns of varying spatial configuration (but of constant total luminance) remarkable changes of amplitude and latency were observed in the ERG b-wave.[103]

It has been accepted for some time that the inner retinal layers (which would be the obvious site for a rigorously developed receptive field architecture) do not contribute to ERG activity. Indeed, it is well known that, in patients with optic nerve pathology, the ERGs elicited by a diffuse flash are unaffected. However, recent studies have shown that pattern ERGs are markedly disturbed after experimental sectioning of the cat optic nerve.[104] This new finding, if confirmed in humans with optic nerve disease, may provide a new diagnostic application for the ERG.

In recent years, the visually evoked potential (VEP) recorded from the scalp overlying the occipital cortex has been established as an excellent, although indirect, measure of foveal function.[105] The power of the VEP to evaluate the fovea is directly related to the manner in which the stimuli are manipulated. Varying the spatial distribution of patterned targets as well as their contrast and temporal properties are common methods of VEP analysis. Because pattern ERGs or those produced with the stimulator ophthalmoscope can provide a direct measure of foveal activity, there is now opportunity for recording evoked electrical activity simultaneously from the retina and cortex using the same stimulus.

RESEARCH NEEDS AND OPPORTUNITIES

Diagnostic retinal research has slowly developed from modest beginnings, but it is beginning to come into its own as part of the overall National Eye Institute program. The following research priorities support the development of clinically applicable noninvasive ocular tests and evaluations of visual function and need to be pursued. Related research priorities in other subprograms can be found elsewhere in this report. The research opportunities raised here are truly cross-cutting in nature and will in large measure depend on multidisciplinary skills and talents for their realization.

Training programs must be strengthened and expanded. The goal is to train researchers in the basic sciences to apply their skills to clinical problems and ophthalmic clinicians to apply basic knowledge more effectively. A broad range of skills and knowledge is needed to train scientists in this area. Essentially three education steps are required. First, the trainee-scientist must become knowledgeable in a scientific discipline that will prepare him or her to make a substantive contribution to the field, for example, some branch of optics, psychophysics, or physiology. Second, the individual needs to gain an in-depth understanding of the structure and function of the eye and the visual system. Finally, he or she needs to have an orientation to clinical eye studies to appreciate existing problems and scientific opportunities. Scientists enter this area of scientific specialization from either basic or clinical backgrounds. Generally, the point of entry defines the scientific interest of the individual in later years. Many members of this group obtain positions in ophthalmological or optometric research programs. Clinicians in training must be able to maintain their skills and participate in shorter, more intense programs. In many instances, success results from effective interaction of the members of a clinician/basic scientist team, each of whom has knowledge which complements the others' expertise.

RECOMMENDATIONS

Based on the foregoing assessment of recent accomplishments, current activities, and research needs and opportunities in "Noninvasive Techniques in the Study of Retinal Disorders," the Panel has made the following recommendations concerning research in this subprogram over the next five years. These have all been designated as Program Development Priorities and include areas of ongoing research in which new knowledge and techniques offer particular opportunities for scientific progress, or promising new areas of research in which there is little or no support at present but where there is both great need and high potential for success. Such areas are judged to warrant significantly increased support over the next five years, provided that high quality applications for research grants in these areas are forthcoming.

Program Development Priorities

- Refine and further develop practical instrumentation for measuring local (focal) ERG and VER and for correlating responses from local ERG

and cortical VEP measurements (see Chapter 10 and *Volume Two, Part Five, Report of the Strabismus, Amblyopia, and Visual Processing Panel*).

- Develop improved noninvasive tests to assess retinal function and localize retinal defects in diseases of the retina and choroid.
- Develop reliable noninvasive techniques for assessing retinal function in infants (see *Volume Two, Part Five, Report of the Strabismus, Amblyopia, and Visual Processing Panel*).
- Utilize noninvasive techniques in studies of etiology, pathogenesis, diagnosis, prevention, and treatment of specific retinal disorders as described in other chapters of this report (see Chapters 1, 3, 4, 5, 6, "Retinal Detachment and Vitreous Disorders," 8, and 10).
- Improve and better define means of evaluating retinal function in the presence of opaque media.

RESOURCE REQUIREMENTS

After reviewing current research grant support in each of these categories and assessing the need and potential for future development, the Panel has estimated the number of projects it believes are needed to carry out its recommendations in FY 1983. These estimates are shown in the table on the following page. For a discussion of the general basis and significance of these projections, see the "Summary" at the beginning of this report.

RESOURCE TABLE

NONINVASIVE TECHNIQUES IN THE STUDY OF RETINAL DISORDERS*

	No. of Grants FY 1981	Panel Recommendation FY 83	
		Add. Grants	Total Grants
Program Development Priorities			
A. Refine and further develop practical instrumentation for measuring local (focal) ERG and VER and for correlating responses from local ERG and cortical VEP measurements.	2	2	4
B. Develop improved noninvasive tests to assess retinal function and localize retinal defects in disease.	8	4	12
C. Develop reliable noninvasive techniques for assessing retinal function in infants.	**	**	**
D. Utilize noninvasive techniques in studies of etiology, pathogenesis, diagnosis, prevention, and treatment of retinal disorders.	*	*	*
E. Improve and better define means of evaluating retinal function in the presence of opaque media.	0	2	2
Subtotal Grants	**10**	**8**	**18**
(% of Program)	**(3)**	**(7)**	**(4)**
Total Estimated Cost	**$1,176,000**	**$714,000**	**$1,890,000**

*See also Chapter 1, Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities; Chapter 3, Tumors; Chapter 4, Developmental and Hereditary Disorders; Chapter 5, Macular Degeneration; Chapter 6, Retinal Detachment and Vitreous Disorders; Chapter 8, Retinal Pigment Epithelium; and Chapter 10, Glial Cells and the Retinal Microenvironment.

**See *Volume Two, Part Five, Report of the Strabismus, Amblyopia, and Visual Processing Panel*, Chapter 1, Normal and Abnormal Development and Chapter 3, Amblyopia.

REFERENCES

1. Research Grant Applications sought by the National Eye Institute on Studies of the Human Visual System in Health and Disease Using Modern Techniques of Psychophysics and Physiological Optics. *NIH Guide for Grants and Contracts* 1, No 4, March 10, 1974, pp 1–4.
2. Workshop on the Role of Psychophysics and Physiological Optics in Ophthalmic Diagnosis and Patient Evaluation: Diagnosis and Patient Evaluation. *Adv Ophthalmol* 41:149–216, 1980.
3. Pomerantzeff O: A lens system for wide-angle fundus photography. *Int Ophthalmol Clin* 16:101–108, 1976.
4. Enoch JM (ed): *Perimetric Standards and Perimetric Glossary of the International Council of Ophthalmology*. The Hague, Dr W Junk Publishers, 1979.
5. Isayama Y, Tagami Y: Quantitative maculometry using a new instrument in cases of optic neuropathies. *Doc Ophthalmol* 14:237, 1977.
6. Johnson C, Enoch JM: Human psychophysical analysis of receptive field-like properties: VI. Current summary and analysis of factors affecting the transient-like function. *Doc Ophthalmol* 14:231, 1977.
7. Ohta Y, Miyamoto T, Harasawa K: Experimental fundus photoperimeter and its application. *Doc Ophthalmol* 19:315–318, 1978.
8. Ohta Y, Momonaga M, Miyamoto T, et al: Visual field studies with fundus photoperimeter in postchiasmatic lesions. *Doc Ophthalmol* 26:119–126, 1981.
9. Enoch JM, Fitzgerald CR, Campos EC: *Quantitative Layer-by-Layer Perimetry: An Extended Analysis*. New York, Grune & Stratton, 1980.
10. Ripps H: Rods, rhodopsin, and the visual response, in Proenza L, Enoch JM, Ginsburg A, et al (eds): *Clinical Applications of Visual Science*. New Rochelle, Cambridge University Press, 1981.
11. Calkins J, Leonard C: Holographic recording of a retina using a continuous wave laser. *Invest Ophthalmol Vis Sci* 9:458–462, 1970.
12. Rosen AM: Fundus holography through a wide-angle contact lens. *Invest Ophthalmol Vis Sci* 12:786–788, 1973.
13. Tokuda AR, Auth DC, Brückner AP: Development of a holographic camera for 3-D microscopy of the unanesthetized human eye. *J Opt Soc Am* 68:1382, 1978.
14. Goldmann H, Lotmar W: Rapid detection of changes in the optic disc: Stereo chronoscopy: II. Evaluation technique, influence of some physiological factors, and follow-up of a case of choked disc. *Albrecht Von Graefes Arch Klin Exp Ophthalmol* 205:263–277, 1978.
15. Newman NM: Ophthalmoscopy observation of the retinal nerve fiber layer. *Trans Am Acad Ophthal Otolaryngol* 83:786–796, 1977.
16. Carr RE, Ripps H Siegel IM: Visual pigment kinetics and adaptation in fundus albipunctatus. 11th ISCERG Symposium. *Doc Ophthalmol* 193–199, 1974.
17. Mizuno K, et al: Red-free light fundus photography, photographic optograms. *Invest Ophthalmol Vis Sci* 7:241–248, 1968.
18. Feke GT, Riva EC: Laser Doppler measurements of blood velocity in human retinal vessels. *J Opt Soc Am* 68:526–531, 1978.
19. Riva C, Ben Svia I: Two point fluorophotometer for the human ocular fundus. *Appl Optics* 14:2691–2693, 1975.
20. Coleman DJ: Combined B and A scan ultrasonography in the management and preoperative assessment of vitreo-retinal pathology. *Mod Probl Ophthalmol* 18:12–26, 1977.
21. Coleman DJ, Lizzy FL: Computer-processed acoustic spectral analysis. *Trans Am Acad Ophthalmol Otolaryngol* 83:725–730, 1977.
22. Coleman DJ: Ultrasonic evaluation of the vitreous, in Freeman HM, Hirose T, Schepens DC (eds): *Vitreous Surgery and Advances in Fundus Diognosis and Treatment*. New York, Appleton-Century-Croft, 1977, pp 63–77.
23. Coleman DJ, Lizzy DL, Jack RL: *Ultrasonography of the Eye and Orbit*. Philadelphia, Lea & Febiger, Inc, 1977.
24. Greve EL, Verriest G (eds): Proceedings, Fourth International Visual Field Symposium. *Doc Ophthalmol* 26, 1981.
25. Dr. Hirose of the Retina Foundation in Boston: presentation of an example of the coupling of the wide-angle fundus camera and a variety of electrophysiological measures at the meeting of the Retina Society in Philadelphia in September 1980.
26. Sandberg, MA, Jacobson SG, Berson EL: Foveal cone electroretinograms in retinitis pigmentosa and juvenile macular degeneration. *Am J Ophthalmol* 88:702–707, 1979.
27. Sandberg MA, Ephron MH, Berson EL: Focal cone electroretinograms in dominant retinitis pigmentosa with reduced penetrance. *Invest Ophthalmol Vis Sci* 17:1096–1101, 1978.
28. Ripps H: Night blindness and the retinal mechanisms of visual adaptation. *Ann R Coll Surg Engl* 58:222, 1976.
29. Enoch JM: Quantitative layer-by-layer perimetry. *Invest Ophthalmol Vis Sci* 17:199–257, 1977.
30. Enoch JM, Campos EC: New quantitative perimetric tests designed to evaluate receptive field-like properties in diseases of the retina and the optic nerve, in Solol S (ed): *Electrophysiology and Psychophysics: Their Use in Ophthalmic Diagnosis*. Boston, Little Brown & Co, 1980.
31. Adams AJ, Zisman F, Cavender JC: Sensitivity loss in chromatic processing channels of diabetics. *Invest Ophthalmol Vis Sci* 19(suppl):169, 1980.
32. Zisman F, Adams AJ, Cavender JC: Chromatic, luminosity and contrast sensitivity changes in diabetics. *Invest Ophthalmol Vis Sci* 20:93, 1981.
33. Smith VC, et al: Visual function in acute posterior multifocal placoid pigment epitheliopathy. *Am J Ophthalmol* 85:192–199, 1978.
34. Pokorny S, Smith VC, Diddie K, et al: Color matching and Stiles-Crawford effect in early senile macular degeneration. *Invest Ophthalmol Vis Sci* 16(suppl):160, 1977.

35. Galvin RJ, Regan D, Heron J: Impaired temporal resolution of vision after acute retrobulbar neuritis. *Brain* 99:255–268, 1976.
36. Galvin R, Heron J, Regan D: Subclinical optic neuropathy in multiple sclerosis. *Arch Neurol* 34:666–670, 1979.
37. Smith VC, Pokorny J, Diddie KR: Color matching and Stiles-Crawford effect in central serous choroid pathy. *Mod Probl Ophthalmol* 19:284–295, 1978.
38. Fitzgerald, CR, Birch DG, Enoch JM: Functional analysis of vision in patients after retinal detachment repair. *Arch Ophthalmol* 98:1237–1244, 1980.
39. Fitzgerald CR, et al: Comparison of visual function studies in two cases of senile macular degeneration. *Albrecht Von Graefes Arch Klin Exp Ophthalmol* 210:79–91, 1979.
40. Fitzgerald CR: "Exudative" senile macular degeneration, in Kaufman HE, Zimmerman TJ (eds): *Concepts of Ophthalmology*, ed 5. St Louis, CV Mosby Co, 1976.
41. Campos EC, et al: Retinal receptive field-like properties and Stiles-Crawford effect followed in a patient with a traumatic choroidal rupture. *Doc Ophthalmol* 45:381–395, 1978.
42. Bresnick G, Pokorny J, Smith VC: Effect of traction in diabetic retinopathy. *Am J Optom Physiol Opt*, to be published.
43. Enoch JM, et al: Different functional changes recorded in open-angle glaucoma and anterior ischemic optic neuropathy. *Doc Ophthalmol*, to be published.
44. Enoch JM, et al: Measurement of visual resolution at high luminance levels in patients with possible demyelinating disease. *Int Ophthalmol Clin* 1:99–104, 1979.
45. Fitzgerald CR, Enoch JM, Temme LA: Kinetic perimetry as a sensitive indicator of visual fatigue or saturation-like defects in retrobulbar anomalies. *Doc Ophthalmol* 26:293–303, 1981.
46. Enoch JM, Yamada S, Namba A: Contrast sensitivity functions measured in patients with high refractive errors with emphasis on aphakia. *Doc Ophthalmol* 47:139–162, 1979.
47. Gucukaglu A, Arden GB: Low-frequency contrast sensitivity is reduced in very early macular disease. *Invest Ophthalmol Vis Sci* 17:293, 1978.
48. Sjöstrand J, Frisen L: Contrast sensitivity in macular disease. *Acta Ophthalmol (Copenh)* 55:507, 1977.
49. Atkin A, Bodis-Wollner I, Wolkstein M, et al: Abnormalities of central contrast sensitivity in glaucoma. *Am J Optom Physiol Opt* 88:205–211, 1979.
50. Sekuler R, Muttman LP: Spatial vision and aging: I. Contrast sensitivity. *J Gerontol* 35:692–699, 1980.
51. Arden GB, Jacobson JJ: A simple grating test for contrast sensitivity: Preliminary results indicate value in screening for glaucoma. *Invest Ophthalmol Vis Sci* 17:23, 1978.
52. Hilz R, Rentschler L, Brettol H: Myopic and strabismic amblyopia: Substantial differences in human visual development. *Exp Brain Res* 30:445, 1977.
53. Itoi M, Yamamoto T: Clinical application of modulation transfer function, in *Proceedings of the International Ophthalmology and Optics Symposium*, Tokyo, May 8–9, 1978. Science Council of Japan (Nippon Gakujutsu Kaigi), pp 63–66.
54. Hubel DH, Wiesel TN: The period of susceptibility to the physiological effects of unilateral eye closure in kittens. *J Physiol (Lond)* 206:419–436, 1970.
55. Wiesel TN, Hubel DH: Ordered arrangement of orientation columns in monkeys lacking visual experience. *J Comp Neurol* 158:307–318, 1974.
56. Dobson V, Teller DY: Visual acuity in human infants: A review and comparison of behavioral and electrophysiological studies. *Vision Res* 18:1469–1483, 1978.
57. Teller DY: The forced-choice preferential looking procedure: A psychophysical technique for use with human infants. *Infant Behav Dev* 2:135–153, 1979.
58. Gwiazda J, et al: Infant visual acuity and its meridional variation. *Vision Res* 18:1557–1564, 1978.
59. Leehey SC, et al: Orientation anisotropy in infant vision. *Science* 190:900–901, 1975.
60. Sokol S: Measurement of infant visual acuity from pattern reversal evoked potentials. *Vision Res* 18:33–39, 1978.
61. Marg E, Freeman DN, Peltzman P, et al: Visual acuity development in human infants: Evoked potential measurements. *Invest Ophthalmol Vis Sci* 15:150–153, 1976.
62. Boothe RG, et al: Development of contrast sensitivity in infant *Macaca nemestrina* monkeys. *Science* 208:1290–1292, 1980.
63. Teller DY, Peeples D, Sekel M: Discrimination of chromatic from white light by two-month-old human infants. *Vision Res* 18:41–48, 1978.
64. Fox R, et al: Stereopsis in human infants. *Science* 207:323–324, 1980.
65. Hold R, Birch E, Gwiazda J: Stereoacuity of human infants. *Proc Natl Acad Sci USA* 77:5572–5574, 1980.
66. Laties A: Histochemical techniques for the study of photoreceptor orientation. *Tissue Cell* 1:63–81, 1968.
67. Enoch JM: Retinal receptor orientation and the role of fiber optics in vision. *Am J Optom Physiol Opt* 49:455–470, 1972.
68. Bonds AB, MacLeod DIA: A displaced Stiles-Crawford effect associated with an eccentric pupil. *Invest Ophthalmol Vis Sci* 17:754–761, 1978.
69. Enoch JM, Birch DG, Birch EE: Monocular light exclusion for a period of days reduces directional sensitivity of the human retina. *Science* 206:705–707, 1980.
70. Enoch JM, Birch DG: Inferred positive phototropic activity in human photoreceptors. *Philos Trans R Soc Lond (Biol)* 291:323–351, 1981.
71. Applegate R, Bonds AB: Induced movement of receptor alignment toward a new pupillary aperture. *Invest Ophthalmol Vis Sci*, to be published.
72. Fitzgerald CR, et al: Anomalous pigment epithelial/photoreceptor relationships and receptor orientation. *Invest Ophthalmol Vis Sci* 19:956–966, 1980.
73. Goldmann H, Lotmar W: Beitrag zum Problem der Bestimmung der "retinalen Sehschaerfe" bei Katarakt. *Klin Monatsbl Augenheilkd* 154:324–329, 1969.

74. Green D: Testing of vision of cataract patients by means of laser-generated interference fringes. *Science* 168:1240–1242, 1970.
75. Karpe G: The basis of clinical electroretinography. *Acta Ophthalmol (Suppl) (Copenh)* 24:1, 1945.
76. Goodman G, Ripps H: Electroretinography in the differential diagnosis of visual loss in children. *Arch Ophthalmol* 64:221, 1960.
77. Goodman G, Ripps H, Siegel IM: Cone dysfunction syndromes. *Arch Ophthalmol* 70:214, 1963.
78. Gouras P: Electroretinography: Some basic principles. *Invest Ophthalmol Vis Sci* 9:557, 1970.
79. Berson EL, Gouras P, Hoff M: Temporal aspects of the electroretinogram. *Arch Ophthalmol* 81:207, 1969.
80. Rabin AR, Berson EL: A full-field system for clinical electroretinography. *Arch Ophthalmol* 92:59, 1974.
81. Siegel IM: A ganzfeld contact lens electrode. *Am J Ophthalmol* 80:296, 1975.
82. Berson EL, Gouras P, Gunkel RD, et al: Dominant retinitis pigmentosa with reduced penetrance. *Arch Ophthalmol* 81:224, 1969.
83. Carr RE, Ripps H, Siegel IM, et al: Rhodopsin and the electrical activity of the retina in congenital nightblindness. *Invest Ophthalmol Vis Sci* 5:497, 1966.
84. Berson EL, Rosen JB, Siminoff EA: Electroretinographic testing as an aid in detection of carriers of X-chromosome-linked retinitis pigmentosa. *Am J Ophthalmol* 87:460, 1979.
85. Wachtmeister L: On the oscillatory potentials of the human ERG in light and dark adaptation: IV. Effect of adaptation to short flashes of light. *Acta Ophthalmol (Copenh)* 50:250, 1972.
86. Yonemura D, Tsuzuki K, Aoki T: The clinical importance of oscillatory potentials in the human ERG. *Acta Ophthalmol (Suppl) (Copenh)* 70:115, 1962.
87. Ogden TE: The oscillatory waves of the primate electroretinogram. *Vision Res* 13:1059, 1973.
88. Yonemura D, Aoaki T, Tsuzuki K: Electroretinogram in diabetic retinopathy. *Arch Ophthalmol* 68:19, 1962.
89. Gjotterberg M: The ERG in diabetic retinopathy: A clinical study and a critical survey. *Acta Ophthalmol (Copenh)* 52:521, 1974.
90. Wachtmeister L, Dowling JE: The oscillatory potentials of the mudpuppy retina. *Invest Ophthalmol Vis Sci* 17:1176, 1978.
91. Arden GB, Barrada A, Kelsey JH: New clinical test of retinal function based on the standing potential of the eye. *Br J Ophthalmol* 46:449, 1962.
92. Steinberg RH, Schmidt R, Brown KT: Intracellular responses to light from cat pigment epithelium: Origin of the electroretinogram c-wave. *Nature* 227:728, 1970.
93. Nilsson SEG, Skoog KO: Covariation of the simultaneously recorded c-wave and the standing potential of the human eye. *Acta Ophthalmol (Copenh)* 53:721, 1975.
94. Steinberg RH, Niemeyer G: Light peak of cat DC electroretinogram: Not generated by a change in (K^+). *Invest Ophthalmol Vis Sci* 20:414, 1981.
95. Deutman AF: Electro-oculography in families with vitelliform dystrophy of the fovea. *Arch Ophthalmol* 81:305, 1969.
96. Aiba TS, Alpern M, Maaseidvaag F: The electroretinogram evoked by the excitation of human foveal cones. *J Physiol (Lond)* 189:43, 1967.
97. Sandberg MA, Berson EL, Ariel M: Visually evoked response testing with a stimulator-ophthalmoscope: Macular scars, hereditary macular degeneration and retinitis pigmentosa. *Arch Ophthalmol* 95:1805, 1977.
98. Sandberg MA, Jacobson SG, Berson EL: Foveal cone electroretinograms in retinitis pigmentosa and juvenile macular degeneration. *Am J Ophthalmol* 88:702, 1979.
99. Sokol S: An electrodiagnostic index of macular degeneration. *Arch Ophthalmol* 88:619, 1972.
100. Armington JC: The electroretinogram, the visually evoked potential and the area-luminance relation. *Vision Res* 8:263, 1968.
101. Sokol S, Nadler D: Simultaneous electroretinogram and visually evoked potentials from adult amblyopes in response to a pattern stimulus. *Invest Ophthalmol Vis Sci* 18:848, 1979.
102. Arden GB, Vaegenhogg CR, et al: Pattern ERGs are abnormal in many amblyopes. *Trans Ophthalmol Soc UK*, to be published.
103. Nelson R, Zrenner E, Gouras P: Patterned stimuli reveal spatial organization in the electroretinogram. *Proceedings 16th ISCEV Symposium*, Morioka, Japan, 1978, pp 161–169.
104. Maffei L, Fiorentino A: Electroretinographic responses to alternating gratings before and after section of the optic nerve. *Science* 211:953, 1981.
105. Sokol S: Visually evoked potentials: Theory, techniques, and clinical application. *Surv Ophthalmol* 21:18, 1976.

14

TISSUE ACQUISITION AND DISTRIBUTION: HUMAN DONOR EYES AND ANIMAL MODELS

INTRODUCTION

PROGRESS IN UNDERSTANDING human disease depends to a great extent upon the acquisition and study of appropriate human and animal retinal tissues. Because human biopsy material from the retina and choroid is unavailable to the vision research community, it is essential to obtain for study human eyes taken postmortem or removed because of malignancy and eyes from animals with hereditary retinal diseases.

Evidence suggests that specific biochemical defects may underlie each type of hereditary retinal and choroidal disease. If this is true, biochemical studies of normal and diseased human donor retinas are crucial. Similarly, comparative biochemical studies of retinas from animals afflicted with hereditary retinal degenerations are needed. Indeed, investigations of human and animal retinal disorders should be considered as closely interrelated. The discovery of a specific biochemical defect in animal eyes can pinpoint an area of study applicable to human tissue. Even in the absence of precise overlap in pathogenesis, the study of animal models opens the door to experimental therapeutic trials, which could lead to the development of therapies for human disease. Furthermore, the animals themselves would benefit from discovery of a specific cause for these blinding diseases and effective treatment.

The same reasoning applies to research on the cause and treatment of diabetic retinopathy, uveitis, retinal and choroidal tumors, and retinal detachment, and, in fact, nearly all the major types of retinal and choroidal disorders. In searching for the cause of a disorder, there is no experimental material of greater value than the affected tissue.

Donor eye specimens, to be of full value, must be properly classified premortem and must be processed according to a specific research protocol, because the worth of the donor eye is directly correlated with the degree of documented clinical history of that eye. The more accurate the classification of the disease process and the more complete the documentation of the precise clinical course and status, the greater the research potential of an individual donor eye.

Similarly, the procedure for tissue preservation is important; frequently, this calls for quick freezing and storage at low temperatures or use of special solutions or fixatives. Electron microscopic studies require that tissue be preserved within four hours after death, and preferably within two hours; biochemical investigations frequently require even more rapid processing. If the eye is not so treated, deterioration occurs and the value of the eye is reduced substantially.

The cost and work involved in the acquisition, care, breeding, and distribution of animals with specific pathological conditions may be overwhelming to an individual researcher, and requires a specific program designed for this purpose. That such a program can be mounted and made practical has been demonstrated by the success of the National Eye Institute's contract for breeding and distributing to researchers the Royal College of Surgeons (RCS) rat with hereditary retinal degeneration. Both Irish setters and miniature poodles with

inherited retinal disorders also have been bred successfully at an animal facility which is supported by a National Eye Institute contract.

The need for human and animal tissues for vision research has greatly increased in recent years due, in part, to the tremendous research progress of the last decade. During this time, knowledge of the anatomy, physiology, and biochemistry of the retina and choroid has expanded significantly; and improved methodology has made possible the study of individual cell types with sophisticated instrumentation. Advances in technology have been coupled with an increasing public awareness about diseases of the retina and choroid, in particular, retinitis pigmentosa and allied diseases as well as diabetic retinopathy.

Early detection of retinitis pigmentosa often has alerted older affected relatives to the fact that the condition is familial, and this, in turn, has motivated them to donate their eyes for study after death. Advances in the preservation of corneal tissue have made it possible to use the corneas of many normal postmortem human donor eyes for planned corneal transplants while using the retina and choroid for research, thereby maximizing the use of every donor eye. Eye banks and private foundations having close contacts with patients who are afflicted with retinitis pigmentosa and diabetic retinopathy are aware of this potential and have devoted resources to improving the acquisition and distribution of human donor eyes.

SUBPROGRAM OBJECTIVES

- To make available postmortem human donor eyes, both from normal individuals and those affected by retinal and choroidal diseases, for comparative study by modern techniques.
- To discover and develop animal models of retinal and choroidal diseases.
- To provide a mechanism for breeding promising animal models to provide adequate numbers for distribution to qualified investigators.

OVERVIEW OF CURRENT RESEARCH SUPPORT

Within the past five years, a National Eye Institute contract for developing congenic strains of normal and diseased Royal College of Surgeons rats has been successfully completed, and now many centers in this country are investigating the cause of retinal degeneration in these rats. Colonies of mice with hereditary retinal disease are available through the Jackson Laboratories, Bar Harbor, Maine, and through research centers that maintain colonies of these mice.

A program to supply Irish setters afflicted with rod-cone dysplasia and miniature French poodles with progressive retinal atrophy was initiated in 1975 by the National Retinitis Pigmentosa Foundation and subsequently expanded by the National Eye Institute. Qualified investigators requesting animals are required to submit protocols describing the planned experiments. These protocols are competitively reviewed and animals and tissues are distributed on the basis of merit. This program has already supplied Irish setters to many investigators for biochemical and ultrastructural studies and, more recently, has begun to supply miniature French poodles with progressive retinal atrophy for electrophysiological, ultrastructural, and biochemical studies.

Individual research centers have structured their own donor programs. For example, a uveitis research project is being conducted under the auspices of the University of California School of Medicine in San Francisco, and an eye donor program for patients with retinal degenerations is conducted by several universities throughout the United States. Individual laboratories have cooperated for the most part with their regional eye banks to obtain postmortem human donor eyes. A pilot study sponsored by the National Retinitis Pigmentosa Foundation with the Maryland Eye Bank demonstrated the feasibility of sending normal postmortem human donor eyes to various centers in the United States. It was clear from this pilot effort that the tissue had to be preserved as soon as possible after death, preferably no later than four hours for useful ultrastructural study, and no later than two hours for some biochemical measurements.

The National Retinitis Pigmentosa Foundation has encouraged a collaborative effort among nine centers that it supports to exchange research protocols for use of donor eyes, and is aiding in the registration of donors at various research centers that receive support from the Foundation. The Juvenile Diabetes Foundation has received support to begin a program (the National Diabetes Research Interchange) to ensure acquisition and appropriate distribution of postmortem donor eyes from patients with diabetes mellitus.

With respect to dogs, Irish setters and miniature French poodles with inherited retinal disease have been bred, but no obvious mechanism exists for breeding and distributing other species with retinal degenerations that may become available. Similarly, dogs with diabetic retinopathy exist, but communi-

cation needs to be improved so that more researchers in the United States become aware of them.

RECENT ACCOMPLISHMENTS

Research interest in postmortem normal human donor eyes and eyes from patients with hereditary retinal diseases has increased substantially in the past few years. Retinal pigment epithelial cells from normal donor eyes can be cultured [1-7] as late as 40 hours after death; [6-9] best results are obtained when eyes are removed within five hours after death and placed immediately on ice. [7,9] Cell lines have been developed for subsequent biochemical [5,7-10] and ultrastructural studies. Studies have begun on pigment epithelial cells cultured from postmortem donor eyes of patients with retinitis pigmentosa. [8,9]

The photoreceptors of the normal human retina have been shown to retain a postmortem metabolic capacity for at least four hours after death; [11,12] perhaps the length of time in which metabolic capacity is retained may be extended if the eyes are placed on ice as soon as possible. [12] Functional capacity may also be preserved for longer periods if the incubation media is modified or if the vasculature of the enucleated eye is perfused with an appropriate artificial medium. [13] Photoreceptor cell-specific processes that have been studied include the capacity to synthesize rhodopsin from ^{14}C-labeled amino acids, the high affinity uptake mechanism for ^{3}H-taurine, and the ability to incorporate inorganic ^{32}P-phosphate into rhodopsin with exposure to light. [12] Pericytes [14] and endothelial cells [15,16] from retinal blood vessels have been successfully cultured for study of possible pathogenetic mechanisms in diabetic retinopathy. [17] Postmortem changes and the viability of retinal vessels after cold storage also have been studied. [18] In addition, postmortem human eyes have been used recently to identify the cellular sites of putative amino acid neurotransmitters in the retina. [19]

Hereditary retinal and choroidal diseases have been investigated in the mouse; rat; several strains of dogs including the Alaskan malamute, Irish setter, miniature French poodle, and collie; and strains of baboon and rhesus monkey. Biochemical defects in the photoreceptors have been delineated in the mouse and Irish setter, a defect in the phagocytic capacity of pigment epithelium has been shown in the RCS rat, the degeneration in the baboon has been compared with progressive cone-rod degeneration in man; and the degeneration in the rhesus monkey resembles Doyne's honeycomb choroiditis in man. A strain of rats (BB rat) develops an autoimmune insulitis and consequent diabetes mellitus. [20] Models of diabetic retinopathy have been produced in the dog with alloxan and in the rat with streptozotocin. Progress has been made toward establishing animal models for retinoblastoma and malignant melanoma. [21]

Human donor eyes with retinitis pigmentosa have been studied in several centers in the United States, and clinicopathologic correlations have been made relating premortem clinical findings with postmortem ultrastructural and biochemical changes. [22-32]

RESEARCH NEEDS AND OPPORTUNITIES

There is great need for human and animal tissue to study pathogenetic mechanisms in a variety of eye diseases involving the retina and choroid. With proper organization and public education, a great potential clearly exists to increase the number of postmortem human donor eyes available for study. Opportunities exist for obtaining normal human donor retinas from eye banks all over the United States without compromising existing programs for supplying tissue for corneal transplantation. In families with hereditary retinitis pigmentosa and with diabetes mellitus, increasing awareness of the potential uses of donor eyes has resulted in a larger number of individuals who are willing to donate their eyes after death for research.

There are also increased opportunities, coincident with advances in techniques, to study the cell biology of the diseased cells of animals with hereditary retinal and choroidal disease. Larger numbers of dogs with retinal degenerations and diabetic retinopathy will be needed in the future, particularly when treatment trials are contemplated.

A program for obtaining human donor eyes as soon as possible after death requires a plan for registration of donors and acquisition of tissue. The registration of human donors is handled best through eye banks and private foundations which have close contact with patients. The costs of acquiring the eyes and the expenses related to the study of the eyes once research protocols have been approved by appropriate peer review should be supported by National Eye Institute grants. Eye banks, private foundations, pathologists, and investigators must arrange for 24-hour coverage to insure that all donor eyes are received and prepared promptly and appropriately. Special research protocols may be required for different eyes depending on the extent and type of retinal disease and the investigator, in some instances, may have to be present when the donor eyes are taken.

Once a potential donor has registered, an investigator must obtain as much clinical information as possible about him or her to make future clinicopathological correlations meaningful. Eye banks and private foundations can play another important role by educating the public and assigning prospective donors to a registry in a given region. Greater availability of animal models with hereditary disease will undoubtedly encourage more investigators to study these diseases. A need exists to take advantage of the development of new models by creating adequate facilities to breed them for interested investigators. The expenses related to transportation of animals and housing of animals at a given research center, and the expenses related to the research on these animals, could be supported through the individual project grants.

RECOMMENDATIONS

Based on the foregoing assessment of recent accomplishments, current activities, and research needs and opportunities in "Tissue Acquisition and Distribution: Human Donor Eyes and Animal Models," the Panel has made the following recommendations concerning research in this subprogram over the next five years. These have all been designated as Program Development Priorities and include areas of ongoing research in which new knowledge and techniques offer particular opportunities for scientific progress, or promising new areas of research in which there is little or no support at present but where there is both great need and high potential for success. Such areas are judged to warrant significantly increased support over the next five years, provided that high quality applications for research grants in these areas are forthcoming.

Program Development Priorities

- Procure, prepare, and distribute for study postmortem human donor eyes from those affected with retinal and choroidal diseases. Ultrastructural, biochemical, and tissue culture studies should be conducted with careful attention to obtaining correlations with premortem clinical findings (see Chapter 1, "Diabetic Retinopathy, Sickle Cell Retinopathy, and Other Vascular Abnormalities;" Chapter 2, "Inflammatory Disorders;" Chapter 3, "Tumors;" Chapter 4, "Developmental and Hereditary Disorders;" Chapter 5, "Macular Degeneration; and Chapter 6, "Retinal Detachment and Vitreous Disorders").
- Identify, develop, and maintain new animal models of retinal and choroidal diseases. Particularly promising animal models should be bred and adequate numbers distributed to qualified investigators (see Chapters 1, 2, 3, 4, 5, and 6).

RESOURCE REQUIREMENTS

After reviewing current research grant support in each of these categories and assessing the need and potential for future development, the Panel has estimated the number of projects it believes are needed to carry out its recommendations in FY 1983. These estimates are shown in the table on the following page. For a discussion of the general basis and significance of these projections, see the "Summary" at the beginning of this report.

RESOURCE TABLE

TISSUE ACQUISITION AND DISTRIBUTION:
HUMAN DONOR EYES AND ANIMAL MODELS

	No. of Grants FY 1981	Panel Recommendation FY 83	
		Add. Grants	Total Grants
Program Development Priorities			
A. Procure, prepare, and distribute postmortem human donor eyes affected with retinal and choroidal diseases; conduct ultrastructural, biochemical, and tissue culture studies; obtain correlations with premortem clinical findings.	*	[4]*	[4]*
B. Identify, develop, and maintain new animal models of retinal and choroidal diseases.	*†	[2]*†	[2]*†
Subtotal Grants (% of Program)	*	**[6]***	**[6]***
Total Estimated Cost	—	—	—

* Grants counted elsewhere within Retinal and Choroidal Diseases subprograms.

† Does not include one contract: "Animal Models of Hereditary Retinal Degeneration." (The continuation of the existing NEI breeding colony for animals with inherited retinal disorders is recommended.)

REFERENCES

1. Barishak YR: In vitro behavior of the pigmented cells of the retina and uvea of the adult eye. *Acta Ophthalmol (Copenh)* 38:339–346, 1960.
2. Albert DM, Tso MOM, Rabson AS: In vitro growth of pure cultures of retinal pigment epithelium. *Arch Ophthalmol* 88:63–69, 1972.
3. Mannagh J, Arya DV, Irvine AR: Tissue culture of human retinal pigment epithelium. *Invest Ophthalmol Vis Sci* 12:52–64, 1973.
4. Edwards RB: Synthesis of glycosaminoglycans by cultured human retinal pigment epithelium. *Invest Ophthalmol Vis Sci* 17(suppl):124, 1978.
5. Del Monte MA: Mucopolysaccharide metabolism of cultured human RPE. *Invest Ophthalmol Vis Sci* 18(suppl):154, 1979.
6. Flood MT, Gouras P, Kjeldbye H: Growth characteristics and ultrastructure of human retinal pigment epithelium in vitro. *Invest Ophthalmol Vis Sci* 19:1309–1320, 1980.
7. Edwards RB: Culture of mammalian retinal pigment epithelium and neural retina, in Packer L (ed): *Methods of Enzymology*, Vol 81, Biomembranes, part H. New York, Academic Press, 1982, pp 39–43.
8. Edwards RB: Studies of cultured human retinal pigment epithelium from normal donors and a patient with retinitis pigmentosa. *Proc Int Soc Eye Res* 1:73, 1980.
9. Edwards RB: Glycosaminoglycan synthesis by cultured human retinal pigment epithelium from normal post-mortem donors and a post-mortem donor with retinitis pigmentosa. *Invest Ophthalmol Vis Sci*, in press.
10. Haley JE, Flood MT, Gouras P: Changes of protein patterns in human retinal pigment epithelial cells in vitro. *Invest Ophthalmol Vis Sci* 20(suppl):165, 1981.
11. O'Brien PJ, Muellenberg CG, Bungenberg de Jong JJ: Incorporation of leucine into rhodopsin in isolated bovine retina. *Biochemistry* 11:64–70, 1972.
12. Schmidt SY, Berson EL: Postmortem metabolic capacity of photoreceptor cells in human and rat retinas. *Invest Ophthalmol Vis Sci* 19:1274–1280, 1980.
13. Hoff M, Gouras P: Tolerance of mammalian retina to circulatory arrest, in Pearlman JP (ed): *Documents Ophthalmological Proceedings Series, X ISCERG Symposium*. The Hague, Dr W Junk Publishers, vol 2, pp 57–63, 1973.
14. Buzney SB, Frank RN, Robison WG: Retinal capillaries: Proliferation of mural cells in vitro. *Science* 190:985–986, 1975.
15. Buzney SB, Massicotte SJ: Retinal vessels: Proliferation of endothelium in vitro. *Invest Ophthalmol Vis Sci* 18:1191–1195, 1979.
16. Frank RN, Kinsey VD, Frank KW, et al: In vitro proliferation of endothelial cells from kitten retinal capillaries. *Invest Ophthalmol Vis Sci* 18:1195–1200, 1979.
17. Frank RN: Tissue culture and biochemical studies of retinal microvessels, in Friedman EA, L'Esperance FA (eds): *Diabetic Renal-Retinal Syndromes*. New York, Grune & Stratton, 1980, pp 123–133.
18. Tripathi RC, Tripathi BJ: Postmortem changes and viability of retinal vessels after cold storage: A differential susceptibility of endothelial cells and pericytes. *Exp Eye Res* 28:539–549, 1979.
19. Lam DM, Hollyfield J: Localization of putative amino acid neurotransmitters in the human retina. *Exp Eye Res* 31:729–732, 1980.
20. Nakhooda AF, Like AA, Chappel CI, et al: The spontaneously diabetic Wistar rat. *Diabetes* 26(2):100–112, 1977.
21. Albert DM: Needs for animal models of human diseases of the eye: Induced animal models of human ocular disease with particular consideration of ocular melanoma. *Am J Pathol* 101(suppl):S177–186, 1980.
22. Kolb H, Gouras P: Electron microscopic observations of human retinitis pigmentosa, dominantly inherited. *Invest Ophthalmol Vis Sci* 13:487–498, 1974.
23. Szamier RB, Berson EL: Retinal ultrastructure in advanced retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 16:947–962, 1977.
24. Szamier RB, Berson EL, Klein R, et al: Sex-linked retinitis pigmentosa: Ultrastructure of photoreceptors and pigment epithelium. *Invest Ophthalmol Vis Sci* 18:145–160, 1979.
25. Kalina RE, Bunt AH, Pagon RA: Clinical-ultrastructural study of a retinal dystrophy. *Invest Ophthalmol Vis Sci* 19(suppl):250–251, 1980.
26. Garcia C, Kretzer F, Redburn D: Ultrastructure and autoradiography of retinitis pigmentosa and Usher's syndrome. *Invest Ophthalmol Vis Sci* 21(suppl):41, 1981.
27. Shakib M, Ripps H, MacDonald ED: Retinal degenerative changes in the Laurence-Moon-Biedl syndrome. *Invest Ophthalmol Vis Sci* 21(suppl):122, 1981.
28. Szamier RB, Berson EL: Retinal histopathology in a carrier of X-chromosome-linked retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 22(suppl):74, 1982.
29. Rayborn ME, Moohead LC, Hollyfield JG: A dominantly inherited chorioretinal degeneration resembling sectoral retinitis pigmentosa. I. Ultrastructural studies. *Invest Ophthalmol Vis Sci* 22(suppl):74, 1982.
30. Ulshafer RJ, Tabor G, Frederick JM, et al: A dominantly inherited chorioretinal degeneration resembling sectoral retinitis pigmentosa. II. Metabolic studies and neurotransmitter uptake. *Invest Ophthalmol Vis Sci* 22(suppl):184, 1982.
31. Bridges CDB, Alvarez RA: A dominantly inherited chorioretinal degeneration resembling sectoral retinitis pigmentosa. III. Selective depletion of 11-cis vitamin A. *Invest Ophthalmol Vis Sci* 22(suppl):184, 1982.
32. Steiner AL, Sidarko J, Hollyfield JG: A dominantly inherited chorioretinal degeneration resembling sectoral retinitis pigmentosa. IV. Immunohistochemical studies. *Invest Ophthalmol Vis Sci* 22(suppl):184, 1982.

HV2532
V825
1983
vol. 2
part 1
vision research: a national plan. 1983-1987 report of the National

DATE DUE

HV2532
V825
1983
vol. 2
part 1
vision research: a national plan: 1983-1987 report of the

AMERICAN FOUNDATION FOR THE BLIND
15 WEST 16th STREET
NEW YORK, N.Y. 10011

DISCRIMINATION PROHIBITED: Under provisions of applicable public laws enacted by Congress since 1964, no person in the United States shall, on the grounds of race, color, national origin, handicap, or age, be excluded from participation in, be denied the benefits of, or be subjected to discrimination under any program or activity (or, on the basis of sex, with respect to any education program or activity) receiving Federal financial assistance. In addition, Executive Order 11141 prohibits discrimination on the basis of age by contractors and subcontractors in the performance of Federal contracts, and Executive Order 11246 states that no federally funded contractor may discriminate against any employee or applicant for employment because of race, color, religion, sex, or national origin. Therefore, the National Institutes of Health grants and awards programs, like every program or activity receiving financial assistance from the Department of Health and Human Services, must be operated in compliance with these laws and Executive Orders.

NIH Publication No. 83-2471